

Peripheral Neuropathy and Its Effects on the Diabetic Foot

A thesis submitted for examination of the degree of Doctor of Medicine

Matthew J Young MB BS MRCP(UK)

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Declaration

No portion of the work described in this thesis has been submitted in support of an application for another degree or qualification by myself, or any other person, in this, or any other, university or institute of learning.

Dr Matthew J Young

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Abstract

This thesis describes a number of studies which explore the hypothesis that endoneurial hypoxia is a major component of the pathogenetic mechanisms of diabetic peripheral neuropathy, and that peripheral neuropathy leads to structural changes in the diabetic foot.

Microvascular blood flow and rheology were studied in three age and sex matched groups; diabetic patients with and without neuropathy, and non-diabetic control subjects. Peroneal nerve motor conduction velocity was significantly associated with transcutaneous oxygen tension, $r = 0.6$. $p < 0.001$. No significant differences in rheological parameters were found between non-neuropathic diabetic patients and controls, but significant adverse changes were found in rheological parameters, prostacyclin levels and fibrinolysis, in diabetic patients with neuropathy, in the absence of other complications.

Peroneal nerve motor conduction velocity was measured in 10 non-diabetic and 6 diabetic patients before and after unilateral femoro-popliteal bypass surgery to assess the effect of improving tissue blood flow on nerve function. The contralateral leg served as a control. Restoring tissue oxygenation was associated with significant improvements in peroneal conduction velocity in both non-diabetic and diabetic patients, which may suggest new therapeutic strategies for peripheral neuropathy in man.

The effects of diabetic neuropathy on the foot were examined by a radiographic survey of the prevalence of bone and soft tissue changes in the feet of diabetic patients and normal controls. This demonstrated that medial arterial calcification is significantly associated with peripheral neuropathy, making the use of ankle pressure indices unreliable in neuropathic patients. It also found an higher than previously recognised prevalence of traumatic and Charcot fractures amongst

neuropathic diabetic patients. Further work demonstrated that Charcot patients have a global neurological impairment when compared to matched neuropathic patients without Charcot changes, and significantly reduced bone mineralisation, a possible predisposing factor for the fractures which often initiate the destructive phase of a Charcot joint.

Finally, a new treatment for acute Charcot neuroarthropathy, intravenous Pamidronate, was evaluated, and proved effective in reducing the increased bone turnover, swelling and discomfort associated with the Charcot process.

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Section One

Introduction and General Methods

Chapter One

Introduction

The Pathogenesis of Diabetic Peripheral Neuropathy

Introduction

Peripheral sensorimotor neuropathy is one of the commonest of the long term diabetic complications, reported to affect 28.5% of 6487 patients in a survey of the United Kingdom hospital diabetes clinic population (Young M et al. 1993a). Despite the fact that foot problems have been recognised for over a hundred years (Pryce 1887), the considerable morbidity due to neuropathic foot problems in diabetic patients persists (Boulton 1990a), the aetiology of neuropathy remains unclear and no reliable treatment has yet been devised.

Measuring neuropathy

The wide variation in the reported prevalence of peripheral neuropathy, from 0 - 93%, is probably due to the relatively small size of most studies, to differences in the diagnostic criteria employed and to the different methods of patient selection. Epidemiological studies have either examined all patients irrespective of type (Goodman et al. 1953, Pirart 1979, Knuiman et al. 1986, Maser et al. 1989), or concentrate on a specific patient group; Type 1 patients (DCCT 1988); Type 2 patients (Lehtinen et al. 1989, Franklin et al. 1990); insulin treated patients (Boulton et al. 1985); patients attending hospital clinics (Young M et al. 1993a). Individual studies have employed different criteria in the definition of neuropathy and whilst some authors have considered painful symptoms alone to be diagnostic (Goodman et al. 1953), others have required signs of nerve dysfunction (Pirart 1979).

The diagnosis of peripheral sensorimotor neuropathy has been made on the basis of symptoms (Franklin et al. 1990), signs (DCCT 1988, Dyck et al. 1991), quantitative sensory tests (Steiness 1957, Bertlesmann et al. 1985) or electrodiagnostic studies (Gregerson 1967). The consensus statement of the San Antonio conference on peripheral neuropathy (1988) recommended that an abnormality of one measure in each of these categories should be present before the diagnosis can be assured. Dyck et al. (1985) proposed that abnormalities in two of three criteria were sufficient to diagnose peripheral sensorimotor neuropathy. The suggested categories were signs, quantitative sensory testing and neurophysiological tests. Neuropathic symptoms are generally held to be too variable to be used reliably (Attali et al. 1990).

The definitive test of neuropathy is still sural nerve biopsy (Veves et al. 1992a). However, as this is an invasive test, it is restricted to specialised research uses, particularly therapeutic trials of potential treatments for diabetic peripheral neuropathy, or where the diagnosis is unclear (Veves et al. 1992a) and other measures

of peripheral nerve function are usually used to diagnose and assess diabetic neuropathy in routine clinical and most research studies.

Peripheral neuropathy can be painful or painless and is recognised clinically by reduced sensation and / or absent or reduced ankle and knee reflexes, which may be accompanied by dysaesthesia or paraesthesia. Clinical scoring systems using these criteria have been employed in the diagnosis of clinical neuropathy for epidemiological surveys, and appear to correlate with quantitative sensory tests (Knuiman et al. 1986, DCCT 1988, Franklin et al. 1990, Young M et al. 1993a). Dyck et al. (1985) reported that clinical scoring systems can correlate with the results of histopathological examination of nerve biopsies from neuropathic diabetic patients but this is not a universal finding (Veves et al. 1991a). Quantitative sensory testing can be used in addition to clinical scoring systems to provide numerical data for trial or clinical records.

Vibration perception thresholds were found to be raised in diabetic patients by Steiness (1957). Subsequent studies have shown an age-related increase in vibration perception threshold in healthy subjects (Bloom et al. 1984, Wiles et al. 1991) in keeping with the decrease in peripheral sensation and loss of ankle reflexes in the elderly (Mayne 1965). A rise in vibration perception threshold has been described with increasing duration of diabetes (Young M et al. 1993a, 1993b) in keeping with the higher prevalence of peripheral neuropathy in patients with a long duration of diabetes (Pirart 1979, Knuiman et al. 1986, Franklin et al. 1990). A vibration perception threshold of greater than 25V has been shown cross-sectionally (Boulton et al. 1986), and more recently prospectively, to be associated with foot ulceration in diabetic patients, carrying a seven-fold increase in risk of ulceration over four years (Young M et al. 1992a).

Thermal perception thresholds (Bertlesmann et al. 1985) are used to test small unmyelinated fibre function in peripheral nerves. However, even using a forced choice

protocol, they have a wide coefficient of variation (Attali et al. 1990) and therefore are generally used to complement other tests of nerve function.

Current perception thresholds (Masson et al. 1989a) and pressure perception threshold (Kumar et al. 1991) have also been used to quantify peripheral neuropathy. Pressure perception has been used extensively in the screening for neuropathy due to leprosy (Birke and Sims 1986) and may be ideally suited to rapid screening for diabetic patients at risk of foot ulceration (Kumar et al. 1991), but neither it nor current perception threshold are widely used in the diagnosis of diabetic neuropathy.

Nerve conduction velocities are the most reliable measure of nerve function (Dyck et al. 1985, Attali et al. 1990). Nerve conduction velocities measure the conduction speed of the largest and fastest fibres within a nerve (Behse et al. 1977) and do not rely on the co-operation of the patient, a significant advantage over quantitative sensory tests, which improves their intra-subject co-efficient of variation (Attali et al. 1990). They have been shown to correlate with the mean fibre density in sural nerve biopsies from diabetic patients with mild peripheral neuropathy (Behse et al. 1977, Sima et al. 1992, Veves et al. 1991a). A low peroneal conduction velocity is associated with the risk of foot ulceration (Young R et al. 1986). Peroneal nerve motor conduction velocity has been shown to decline with age and duration of diabetes (Gregerson 1967) in keeping with other tests of neuropathy, and, as the sural sensory nerve action potential is frequently absent in diabetic neuropathy (Young R et al. 1986), is probably the 'gold standard' of non-invasive tests for quantifying peripheral neuropathy.

The pathogenesis of diabetic peripheral neuropathy

If diabetic neuropathy affected all patients with diabetes then hyperglycaemia alone might be a satisfactory explanation for its development. However, using careful clinical criteria, the prevalence of diabetic peripheral neuropathy is usually less than one third of all patients (Young M et al 1993a) and therefore other factors must play a role. The pathogenesis of diabetic peripheral neuropathy is however, the subject of considerable debate, and whilst other pathogenetic mechanisms, including immunological mechanisms (Rabinowe et al. 1989), and those relating to the presence or absence of nerve growth factors (Levi-Montalcini 1987), have been proposed in the pathogenesis of diabetic peripheral neuropathy, the two main candidates are metabolic and vascular mechanisms. In general, opinion is divided between those who believe that metabolic abnormalities (Clements and Bell et al. 1982, Bays and Pfeifer et al. 1988), including those of the polyol pathway (Greene et al. 1988), are the principal cause of diabetic peripheral neuropathy, and those who favour microvascular disease as its cause (Low 1987, Dyck 1989). Within an individual however, both mechanisms are likely to be at work, and indeed, the two may be related (Low 1987, Bays and Pfeifer et al. 1988). Osmotic changes due to sorbitol accumulation have been demonstrated to cause nerve swelling, which may increase the inter-capillary distance, resulting in endoneurial hypoxia (Griffey et al. 1988), and increases in erythrocyte sorbitol are reported to result in stiff red blood cells (Robey et al. 1987), reducing capillary perfusion.

Metabolic theories of neuropathy

In experimental diabetes, streptozotocin rats develop reduced nerve conduction velocities after four weeks of hyperglycaemia (Cameron et al. 1989, 1991, 1992a,b,c, Eliason 1964, Greene et al. 1975). Nerve swelling and increased hydration have also been noted on pathological examination of peripheral nerve. Histochemical analysis of nerve biopsy specimens from experimental diabetic rats revealed sorbitol accumulation and reduced levels of myo-inositol (Stewart et al. 1967, Gabbay 1975, Greene et al. 1975, 1984, 1988). This led to the main metabolic theory of diabetic neuropathy in which hyperglycaemia leads to excess glucose in the nerve axon, which is then converted to sorbitol via the pentose shunt. For a number of years this accumulation of sorbitol was believed to be accompanied by a reduction in the availability of myo-inositol phosphate which is required for cell membrane repair and ATPase activity (Greene et al. 1984, 1988), but this concept has recently been discounted by many workers in peripheral neuropathy, as, although there is evidence of some improvement in nerve conduction with myo-inositol supplementation in experimental animals (Greene et al. 1975), there appears to be no benefit in man (Gregerson et al. 1983), and normal levels of myo-inositol have been found in biopsy specimens from diabetic patients with neuropathy (Dyck et al. 1980, Hale et al. 1987), despite elevated levels of polyol metabolites (Dyck et al. 1988). The rate limiting enzyme of the polyol pathway is aldose reductase and a number of aldose reductase inhibitors have been developed (Masson and Boulton 1990a). Despite the initial success of these compounds in animals, they have proved to be of limited benefit in established neuropathy in man (Boulton et al. 1990b). This is probably due to the primary difference in the nature of experimental neuropathy and neuropathy in human diabetes. In experimental diabetes the animal usually has uncontrolled, or poorly controlled hyperglycaemia over a relatively short time. Although nerve fibre loss does occur, particularly late on in the course of experimental neuropathy (Sima et al. 1988), metabolic/toxic effects play the major role in any abnormality in the first few weeks,

this is probably similar to the reversible nerve conduction slowing noted in newly diagnosed diabetic patients (Ward J et al. 1971). Even in experimental animals the aldose reductase inhibitors have proved relatively ineffective in reversing established nerve conduction slowing to normal, and are more effective in prevention studies.

In established diabetic neuropathy in man there is usually marked loss of nerve fibres, particularly the myelinated fibres (Malik et al. 1989). Attempts to reverse this pathological defect pharmaceutically are unlikely to be successful (Fagius et al. 1985), although the decline in conduction velocity with increasing duration of diabetes, and age, may be slowed (Boulton et al. 1986). A recent trial in early neuropathy has proved more promising with some improvement in nerve conduction velocities and symptoms (Giugliamo et al. 1993).

Thus, other explanations for the development of diabetic peripheral neuropathy have been sought. In particular, there has been considerable research effort to demonstrate a microvascular component to peripheral neuropathy. Such a component would be in keeping with the microvascular hypotheses for retinopathy (Patel et al. 1992) and nephropathy (Parving et al. 1983), providing an unified theory of diabetic complications. This hypothesis has been given recent support by the finding of increased capillary leakage of albumin, one of the hallmarks of early retinopathy or nephropathy, in patients with peripheral neuropathy alone (Valensi 1991).

The microvascular theory of neuropathy

Fagerberg (1959) described endoneurial abnormalities on histological examination of sural nerves. The changes in capillaries, including endoneurial basement membrane thickening paralleled the clinical severity of diabetic peripheral neuropathy. More recent studies including those of Malik et al. (1989) and Veves et al. (1991a) have also found correlations with microangiopathic changes and nerve dysfunction.

The loss of nerve function following interruption of the blood supply is termed ischaemic conduction failure (Low et al. 1987). This is usually tested in man by applying a sphygmomanometer cuff around the limb to be tested and inflating above systolic blood pressure and measuring nerve function at timed intervals. Measuring the reduction in vibration perception using this technique, Steiness (1959) reported that diabetic patients with peripheral neuropathy demonstrated resistance to ischaemic conduction failure and suggested that endoneurial hypoxia might be responsible for this phenomenon. This 'resistance to ischaemic conduction failure' has been described as one of the hallmarks of diabetic neuropathy and a significant pointer to a role for endoneurial hypoxia in the pathogenesis of diabetic neuropathy (Low et al. 1987). Since then, the evidence that microvascular flow and endoneurial hypoxia are implicated in the development of peripheral neuropathy is increasing in both animal and human studies.

Direct measurement of endoneurial oxygen tension has demonstrated that this is reduced in chronically hyperglycaemic rats, and oxygen supplementation in hyperbaric chambers can ameliorate the fall in nerve conduction velocities that usually occurs (Low et al. 1987). Non-diabetic rats kept in a low oxygen environment have also been shown to develop a peripheral neuropathy (Low et al. 1986). Experimental assessments of nerve blood flow have confirmed that this too is reduced in diabetic rats (Cameron et al. 1992a), and recent research has also demonstrated that a

surgically created proximal arteriovenous shunt can significantly reduce nerve function in the rat hind limb by reducing distal blood flow (Sladky et al. 1991). Studies by Cameron et al. and others have shown that experimental neuropathy can be prevented in streptozotocin rats by treatment with vasodilators from the induction of diabetes (Cameron et al. 1992b). Repeated muscle stimulation with electric shocks has been shown to improve angiogenesis and ameliorate the decline in nerve conduction seen in experimental animals (Cameron et al. 1989). Angiotensin converting enzyme inhibitors may also be able to prevent the development of conduction velocity slowing in experimental diabetes (Cameron et al. 1992b)

In man, direct measurement of endoneurial oxygen tension has also shown that the diabetic sural nerve is hypoxic compared to non-diabetic controls and that the sural oxygen tension was lower than that of the overlying vein (Newrick et al. 1986). The histopathological features of biopsies of sural nerve taken from neuropathic diabetic patients are those suggestive of a microangiopathy (Malik et al. 1989). The main features are patchy fibre loss, which is predominantly myelinated; capillary closure; basement membrane thickening and large spaces between capillaries, which may be widened by endoneurial oedema, thus increasing the diffusion gradient for oxygen. The increased water content of nerves in diabetic patients that has been demonstrated on magnetic resonance imaging may indirectly lend support to this concept (Griffey et al. 1988).

It could be reasoned that if diabetic peripheral neuropathy is due to reduced endoneurial blood flow or hypoxia then a direct reduction in blood flow or impaired tissue oxygenation in non-diabetic patients should result in peripheral nerve dysfunction and the same histological features as those found in diabetes. Malik et al. reported that microangiopathy and myelinated fibre loss can be also be found in biopsies from non-diabetic hypoxic chronic obstructive airways disease patients (Malik et al. 1990), up to twenty percent of whom may develop a peripheral neuropathy

(Nowak et al. 1990). These patients also show the same electrophysiological features as diabetic patients, including resistance to ischaemic conduction failure (Masson et al. 1988). In addition, a reduction in motor conduction velocity (Hunter et al. 1988) and morphological abnormalities, including a reduction in myelinated fibre density have been reported in patients with peripheral vascular disease (Rodriguez-Sanchez et al. 1991). Epidemiologically, peripheral neuropathy has been found to be associated with peripheral vascular disease (Maser et al. 1989) and clinical assessments of peripheral vascular disease have been shown to correlate with peripheral nerve function (Ram et al. 1991). In addition, the creation of arteriovenous shunts for haemodialysis has been demonstrated to lead to a distal neuropathy in a number of case reports (Wilbourn et al. 1983, Knezevic and Mastalgia 1984, Riggs et al. 1989).

Tesfaye et al. (1992a) demonstrated that nerve conduction velocity fails to increase in diabetic patients with neuropathy following exercise, suggesting that there is an impairment of neural blood flow. In this study, diabetic patients with no evidence of neuropathy, diabetic patients with neuropathy and healthy controls performed exercise on a treadmill to their maximum capacity. Whilst the mean peroneal nerve motor conduction velocity of the non-neuropathic diabetic and non-diabetic subjects increased following exercise, and all three groups demonstrated an increase in skin temperature, suggesting an increase in limb blood flow, the neuropathic diabetic patients showed no increase in peroneal conduction velocity. This was thought to show that endoneurial blood flow was impaired and is in keeping with the measurements of Newrick et al. (1986) and the experimental work described above.

Tesfaye et al., by direct television microscopy of the epineurial vessels at open nerve biopsy have reported that the epineurial veins are larger in diabetic neuropathic patients when compared to controls (Tesfaye et al. 1990). When fluorescein is injected into the epineurial arteries it appears in the epineurial veins earlier in the diabetic neuropathic patients suggesting increased arterio-venous shunting in the

nerves of neuropathic patients. This work confirms, at the level of the nerve, earlier work by Boulton et al. (1982) which demonstrated that the oxygen tension in pedal veins was increased to levels above those found within the nerves, again suggesting arterio-venous shunting. In addition, the findings of Partsch (1978), that lower amounts of radioactive labelled albumin microspheres are trapped in the capillary beds of the foot, adds further support to the concept of direct arterio-venous channels in the microvasculature of neuropathic diabetic patients which bypass the nutritive supply.

The reports of increased flow in the medium sized vessels of the foot are in keeping with the findings of Patel et al. (1992) in patients with diabetic retinopathy. Laser Doppler flowmetry of the branch arteries and veins of the fundus demonstrated an increase flow in diabetic patients with retinopathy. The increase in retinal blood flow was observed cross-sectionally in all grades of retinopathy, except when the eye had been treated by laser photocoagulation / ablation. Patel et al. hypothesised that the increased flow led to capillary damage and eventual capillary closure, which could result in microcirculatory ischaemia, in keeping with Tooke's theories of a haemodynamic component to diabetic microvascular complications (Tooke 1986). Thus, there is further evidence for a unifying microvascular hypothesis for long term diabetic complications.

Assessing the microvascular circulation

From the studies outlined above, there is good evidence for arterio-venous shunting at the level of medium sized vessels. This shunting might be expected to lead to diversion of nutrient blood flow away from the capillary beds. However, a number of direct studies of the microvasculature have contradicted these findings. Tooke et al. have suggested that at rest there is no evidence of reduced nutritive flow with either laser Doppler flowmetry or direct capillary microscopy (Tooke 1986). However, some of the later work by this group has shown that although capillary pressure and flow are initially increased in diabetes there is a later defect in flow due to capillary sclerosis (Flynn and Tooke 1992). Work from this and other groups, has also shown that although the resting microvascular flow is similar in diabetic patients with and without complications, the hyperaemic response is markedly reduced, suggesting damage to the microcirculation (Rayman et al. 1986, Walmsley et al. 1989, Watkins 1992).

Transcutaneous oxygen measurements are widely used in the assessment of the microvascular and macrovascular blood supply to the foot (Hauser et al. 1984, Gaylarde et al. 1988, Pecoraro et al. 1991) and have been described as being more useful than laser Doppler in measuring tissue perfusion. Gaylarde et al. (1988) demonstrated that at 44 °C, transcutaneous oxygen levels were reduced in diabetic patients with peripheral neuropathy when compared with non-neuropathic diabetic patients, who had lower oxygen tensions than non-diabetic controls. The electrode temperature of 44 °C was preferred because this gave a better correlation with arterial oxygen levels due to arterialisation of capillary blood. At 37 °C the transcutaneous oxygen tension was higher in neuropathic patients than non-neuropathic diabetic patients or controls, but at 17 mmHg, the mean oxygen tension was lower than the described limit of tissue viability in patients with foot ulceration (Pecoraro et al. 1991),

and therefore should be interpreted with caution. A study by Stevens et al. (Stevens M et al. 1992a) in four non-diabetic patients with unilateral traumatic neuropathy of the lower limb showed a lower increase in transcutaneous oxygen levels following heating to 44 °C in the affected limb than the contralateral limb. This study would suggest that the lower oxygen tensions are a secondary phenomena due to the neuropathy, but this was only a small study and co-existent arterial damage may have been present in the patients who suffered injuries to the neurovascular bundle. A reduced hyperaemic response has also been noted in diabetic children before the onset of neuropathy (Shore et al. 1991), and on the abdomen of adults with diabetes (Rayman et al. 1986), suggesting that it is that the impaired hyperaemic response is likely to be a primary microvascular defect rather than a secondary neurogenic phenomenon.

Rheological changes in diabetes

Rheology is the study of blood flow and its determinants. Blood flow is inversely proportional to viscosity in the capillary beds of the microvasculature, and therefore the direct measurement of viscosity and its determinants should indicate the likelihood of flow abnormalities. The principal components of whole blood viscosity are the plasma viscosity, haematocrit, red cell deformability, red cell aggregation and the levels of macroprotein molecules, particularly fibrinogen, which, at low shear rates increase red cell aggregation by forming cross links between red blood cells (MacRury and Lowe 1990).

The role of rheological changes in the development of diabetic complications is still unclear. One study has demonstrated there was no difference in measures of rheology in diabetic and non-diabetic patients with renal failure (Gordge 1990), although both had adverse rheological parameters when compared to healthy non-diabetic controls. A study by the Glasgow group (Lowe et al. 1980a) found no difference in whole blood viscosity between male diabetic patients with proliferative retinopathy versus those with minimal or no retinopathy.

The spectrum of debate about the role of rheological factors in neuropathy ranges from the review of Simpson (1988), which concluded that rheological factors were paramount in the pathogenesis of many forms of peripheral neuropathy, to the work of MacRury et al. which, whilst recognising the potential role for rheological changes in the pathogenesis of peripheral neuropathy, has not found significant differences in any of the rheological parameters measured in neuropathic compared to non-neuropathic diabetic patients (MacRury et al. 1991, MacRury et al. 1993a).

Whole blood viscosity

The evidence for increased whole blood viscosity in diabetes is generally accepted (MacRury and Lowe 1990). However most studies have examined whole blood viscosity in patients with established nephropathy (Gordge et al. 1990) (although the changes were similar in diabetic and non-diabetic patients), retinopathy (Lowe et al. 1980a), or in abnormal metabolic states, including diabetic ketoacidosis (Reubi 1953). In contrast with these findings, there are studies to show that whole blood viscosity improves with good diabetic control (Paisey et al. 1980, Leiper et al. 1984). In addition, no increase in whole blood viscosity has been found in patients with background retinopathy when compared to diabetic patients with no evidence of retinopathy (Lowe et al. 1980a) or in diabetic patients with vs. those without microalbuminuria (Jay et al. 1991). Similarly, MacRury et al. (1991) found no difference in whole blood viscosity at high or low shear rates in diabetic patients with and without neuropathy either at native haematocrit or at a standardised haematocrit, although this study also included patients with other microvascular complications in the neuropathy and control groups. In this study neuropathy was diagnosed on a variety of clinical grounds, with conduction velocities only measured in 18 of the total of 29 neuropathic patients and none of the 'non-neuropathic' and non-diabetic controls. The mean peroneal conduction velocity of the neuropathic patients in the study of MacRury et al. was $40.7 \pm 4.3 \text{ ms}^{-1}$, which is only mildly impaired, and above the threshold for many neuropathy intervention studies (Gregerson 1967, Young R et al. 1986). In another study, which examined whole blood viscosity and other rheological parameters in diabetic patients with and without neuropathy, no non-diabetic controls were studied (Ford et al. 1992). In this study the main determinant of neuropathic status was a peroneal motor conduction velocity of $<40 \text{ ms}^{-1}$. No significant differences were found in whole blood viscosity at high shear rates (230 s^{-1} and 23 s^{-1}) between a group of 'uncomplicated' diabetic patients, although microalbuminuria was not excluded, and neuropathic patients, 11/15 of whom also had other

microvascular complications. In addition, there is no mention of the smoking status of the patients included in either of these studies, and the numbers of type 1 and type 2 patients differ in each group, both of which are believed to influence a number of rheological parameters (Lowe et al. 1980b, MacRury and Lowe 1990).

Haematocrit

Changes in haematocrit have been shown to influence cerebral blood flow in diabetes (Thomas et al. 1977), but not capillary flow in non-diabetic patients with polycythaemia (Tooke and Milligan 1987). An increase in haematocrit increases the amount of red cell aggregation in a cell suspension, particularly at low shear rates (MacRury and Lowe 1990). For this reason, it is usual to correct whole blood viscosity to a standardised haematocrit (Matrai et al. 1987), to reduce the effect that differences in haematocrit between study groups would otherwise have on the measured value of whole blood viscosity.

Red Cell Aggregation

Red cell aggregation is related to haematocrit, fibrinogen concentration and shear rate. It is a major component of whole blood viscosity at low shear rates (MacRury and Lowe 1990). Recently, it has been reported to be increased in diabetic patients with cardiovascular disease (MacRury et al. 1993b), and was also increased in the diabetic subjects in the studies of rheology and peripheral neuropathy (MacRury et al. 1991, 1993a), but again was not significantly different between neuropathic and non-neuropathic diabetic patients.

Plasma viscosity and Fibrinogen

Most recent, widely published studies, confirm that plasma viscosity is increased in diabetic patients, a finding that was first reported by Cogan et al. in 1961. This is generally in association with increased fibrinogen levels in diabetic patients (Auwerx et al. 1988, Ganda 1992, MacRury et al. 1993b). However, other recent studies by MacRury et al. (1991 and 1993a) have contradicted this. One (MacRury et al. 1991) reported an increase in plasma viscosity without an increase in fibrinogen, and the other, no difference in plasma viscosity between diabetic patients and non-diabetic control subjects, although in this study the control group had a very high plasma viscosity (MacRury et al. 1993a).

The relationship between plasma viscosity and increased fibrinogen levels and complications is unclear. Diminno (1986) reported that fibrinogen binding was increased in both diabetic patients with and without retinopathy, but that the increase was only reversed by aspirin in diabetic patients without retinopathy, suggesting that fibrinogen may have a role in retinopathy. The studies of MacRury et al. (1991 and 1993a) and Ford et al. (1992) above reported no differences in either plasma viscosity or fibrinogen levels between diabetic patients with and without neuropathy, although in the study of Ford et al., fibrinogen concentration was strongly associated with increased nerve capillary basement membrane thickness, one of the hallmarks of microangiopathy (Williamson 1977). Therefore, the role of fibrinogen in microvascular complications remains to be clarified.

Red cell deformability

The small nutritive capillaries are on average 3-5 μm in diameter, which compares with a mean red blood cell resting diameter of 8 μm . Therefore, in order to deliver oxygen within capillary beds the red cells have to deform and elongate. Most methods to determine red cell deformability depend on the filtration of red blood cells through a micropore membrane (Dormandy et al. 1985). Again, decreased red blood cell deformability has been reported between groups of diabetic patients and non-diabetic controls in the majority of studies (Kamada and Otsuji 1983, MacRury et al. 1991) although this is not universal (Williamson et al. 1985).

The defect in red cell membrane fluidity has also been reported in experimental diabetes in rats and has been shown to be partly reversible by aldose reductase inhibition. Fractionation of red cells into young and old populations has shown that the decrease in red cell deformability occurs soon after production (Kamada 1992). Glycation of the red blood cell membrane, without a significant change in deformability, has also been reported (Williamson et al. 1985).

The relationship of red cell deformability with diabetic microvascular complications is also unclear.

The functional relevance of rheological changes

The microcirculatory consequences of the changes in rheological parameters that occur in diabetes have not yet been fully established. Such changes may contribute to the lower 'maximal' microvascular blood flow, as measured by transcutaneous oxygen (Gaylarde et al. 1988) or laser Doppler (Rayman et al. 1986, Walmsley et al. 1989, Watkins 1992) reported in patients with diabetic neuropathy. In one of the few studies to examine tissue oxygenation and rheological parameters in diabetic patients, MacRury et al. (1993b) demonstrated that transcutaneous oxygen and whole blood viscosity were negatively correlated. Whole blood viscosity is believed to be increased in diabetic patients (MacRury and Lowe 1990), and therefore this may lead to a reduction in tissue oxygenation in diabetic patients. In a small study of non-diabetic patients with very high whole blood and plasma viscosities due to polycythaemia or Waldenstrom's macroglobulinaemia, capillary blood flow was reduced, but not significantly, when measured directly with television microscopy (Tooke and Milligan 1987). However, the authors suggest that the capillary circulation is able to auto-regulate to accommodate for these changes. Whilst this is true in non-diabetic patients, work from Tooke's unit suggests that auto-regulation in the nail-fold capillaries is lost in diabetic patients soon after diagnosis (Sandeman et al. 1992). Therefore, if changes in viscosity and deformability do occur in diabetes, and are more marked in association with microvascular complications, they may contribute to the increased capillary pressure measured in diabetic patients. This in turn may lead to the endothelial damage, basement membrane changes and increased capillary transudation of albumin that are the hallmarks of diabetic microangiopathy (Williamson et al. 1977). Indirect evidence for this may also be found in the study of Ford et al. (1992), although only three of the 10 patient non-neuropathic group, compared to all of the fifteen neuropathic patients, had histopathological examination

of sural nerve biopsy specimens, significant correlations were found between basement membrane thickening and a number of determinants of microvascular flow.

Platelets, prostacyclin and thromboxane

The balance of platelet aggregation and the patency of the microcirculation are believed to be regulated by the balance of thromboxane (TXB₂) and the prostanoids, PGE₁ and prostacyclin (Moncada and Vane 1979). There is evidence in experimental diabetes that the balance of thromboxane and prostacyclins is disturbed, with an excess of thromboxane synthesis (Gerrard et al. 1980, Karpen et al. 1982) and this has also been reported in man (Halushka et al. 1981). When this reported increase in thromboxane synthesis is taken together with evidence of increased thromboxane receptors on platelets in patients with type 1 diabetes (Modesti et al 1991) this may partially explain the abnormal platelet function in diabetes (Colwell et al. 1978), and the significantly increased platelet aggregation reported in association with microvascular complications (O'Malley et al. 1975, Dallinger et al 1987, O'Donnell et al. 1991).

Prostacyclin synthesis has also been reported to be reduced in animals with experimental diabetes (Ward K et al. 1989) and diabetic patients (Johnson et al. 1979, Silberkane et al. 1979, Ylikorkala et al. 1981). Treatment with prostaglandin E₁ and its analogues, which have a similar antiplatelet aggregation and vasodilatory function to prostacyclin, has been shown to improve conduction velocity in experimental diabetes (Ohno et al. 1992).

The balance of prostaglandin and thromboxane synthesis may be influenced by the balance of dihomogamma-linolenic acid and arachidonic acid (Jamal 1990). The block in the synthesis of gamma-linolenic acid described in diabetes has been reported to be greater in patients with microvascular complications (Jones et al. 1983). Supplementing the diet with dihomogamma-linolenic acid has been shown to reduce platelet aggregation (Kernoff et al. 1977), but the levels of prostanoids were not measured in this study.

In experimental diabetic neuropathy, dietary supplementation with gamma-linolenic acid can prevent the decline in conduction velocity that develops in streptozotocin rats (Cameron et al. 1991, Stevens E et al. 1993). The study of Stevens et al. also demonstrated that endoneurial laser Doppler flux was reduced in untreated animals, but that this was maintained in rats given dietary supplementation with evening primrose oil. Their data also suggested that evening primrose oil reversed resistance to ischaemic conduction block (Stevens E et al. 1993). Two placebo controlled trials of gamma-linolenic acid in the treatment of diabetic peripheral neuropathy in man have now shown small but definite improvements in symptoms, signs and neurophysiological tests (Jamal and Carmichael 1990, Keen et al. 1993). These studies lend further weight to the hypothesis that microvascular and rheological abnormalities may be involved in the pathogenesis of peripheral neuropathy in diabetes.

Fibrinolytic parameters in diabetes

In vivo, the balance between coagulation and fibrinolysis is carefully maintained. Although the majority of studies suggest that there is an hypercoagulable state and a defect in fibrinolysis in diabetes, the small size of some studies (Wieczorek et al. 1993), the failure to define adequately the patient groups, with a mix of different complications and levels of complications, and the failure to control for smoking habit have further confused this question.

The increased levels of von Willebrand factor (Dornan et al. 1983), and fibrinogen (Auwerx et al. 1988, Ganda and Arkin 1992, MacRury et al. 1993a) reported in diabetes are thought to increase the risk of macrovascular disease, particular seen in patients with type 2 diabetes (Morrish et al. 1991). However, hypercoagulability and decreased fibrinolysis might also influence microvascular flow, and in particular might explain the closed capillaries seen in sural nerve biopsies from patients with diabetic peripheral neuropathy (Simpson 1988). Supportive evidence for Simpson's suggestion may be found in the study of Ford et al. (1992), in which capillary lumen size correlated with fibrinolytic activity.

The main activators of the fibrinolytic system are tissue plasminogen activator (t-PA) and urokinase-like plasminogen activator (u-PA). Circulating t-PA is produced by the vascular endothelium; u-PA is produced by the liver and a number of other cell lines. There are two plasminogen activator inhibitors, PAI-1 and PAI-2. PAI-1 is found in plasma, hepatocytes, endothelial cells and platelets. PAI-2 is produced in the trophoblastic epithelium of the placenta and is the main form of plasminogen activator inhibition in pregnancy. Previous studies that have examined levels of t-PA and PAI-1 antigen may not have provided a full picture of the balance of fibrinolysis as the majority of t-PA and its target plasminogen are bound to plasmin when a clot forms, and are then protected from inhibition by circulating PAI-1 (Grant 1991).

Overall, the fibrinolytic system is believed to be impaired in patients with diabetes (Small et al. 1987). It has been suggested that high insulin levels, either due to resistance or pharmacological treatment, are associated with increased PAI-1 levels (Juhan-Vaque 1989, 1991). However, a number of euglycaemic clamp and related studies of non-insulin resistant patients have failed to show such a relationship and obesity and hypertension may be the main correlates of PAI-1 activity (Auwerx 1988, Grant and Metcalf 1990, Potter van Loon 1990, Landin 1991). The in vitro culture of hepatocytes in high concentrations of insulin has however been shown to induce PAI-1 synthesis. Poor diabetes control has also been shown to reduce fibrinolytic activity (Small et al. 1987).

In type 1 diabetes there are reports of increased, unchanged and decreased basal fibrinolytic activity based on studies of the euglobulin clot lysis time, a global parameter of fibrinolysis and levels of t-PA and PAI-1 antigen (Fisher et al. 1991). Studies measuring the activity of each of these parameters have shown lower t-PA or t-PA / PAI-1 activity ratios in type 1 diabetes compared to controls (Auwerx et al. 1988, Fisher et al. 1991, Wieczorek et al. 1993). Wieczorek et al. also demonstrated that there was an attenuated fibrinolytic response following either venous occlusion or insulin induced hypoglycaemia in type 1 diabetes. They suggested that this abnormality in fibrinolysis might be due to an underlying endothelial defect. A reduced response to venous occlusion in type 1 diabetes has also been noted in patients with neuropathy and retinopathy (Walmsley et al. 1991) and retinopathy alone (McLaren et al. 1990, Walmsley et al. 1991). Thus, despite the debate about the basal level of fibrinolysis in patients with type 1 diabetes, there is general agreement that the fibrinolytic response to stimulation is attenuated, and that this deficit may be exaggerated in type 1 patients with complications.

In type 2 diabetes there is a consensus that fibrinolytic activity is reduced, whether clinically without complications (Fuller et al. 1979, Schneider et al. 1988,

Grant 1991, Walmsley et al 1991), or in association with retinopathy (Garcia Frade et al. 1990).

Overall in diabetes fibrinolysis appears to be reduced basally and markedly impaired following stimulation, such as venous occlusion. This pattern has largely been associated with macrovascular disease (Hamsten et al. 1985, Meade et al. 1980), but, as outlined above, has also been found in association with microvascular complications. As yet no study has looked at the fibrinolytic system in diabetic patients with neuropathy in the absence of other complications. The study of Ford et al. (1992) used fibronectin release as its measure of fibrinolysis. This study showed that in mixed groups of type 1 and type 2 patients, fibronectin release, and by association fibrinolysis, was reduced after venous occlusion in patients with peripheral neuropathy.

Summary

In summary, there appears to be a consensus that microvascular flow is abnormal in diabetic peripheral neuropathy. The histological features found on sural nerve biopsy, including capillary basement membrane thickening, are also found in skin biopsies and in the kidneys suggesting generalised microvascular disease. Rheological parameters and fibrinolysis are adversely affected by diabetes, and although these changes may be greater in diabetic patients with microvascular complications this has not been demonstrated in well controlled studies of patients with single complications, such as diabetic neuropathy alone. There is potential for possible interaction between abnormal capillary compliance and size, increased arterio-venous shunting, and the effects of individual adverse rheological parameters to reduce microvascular flow. Such an interaction might explain some of the functional changes in tissue oxygenation and laser Doppler flow described in diabetes. However, to date there is only patchy evidence to support this hypothesis.

Supporting evidence for the role of microvascular abnormalities and endoneurial hypoxia in the pathogenesis of diabetic peripheral neuropathy includes extensive animal work, and evidence of similar histological and clinical findings in patients with respiratory and peripheral vascular diseases. However, although reported in rat models of neuropathy, the reversal of conduction velocity abnormalities by improving tissue oxygenation has not been demonstrated in man.

The Effects of Peripheral Neuropathy on the Diabetic Foot

Introduction

A greater understanding of the pathogenesis of diabetic peripheral neuropathy may help to develop treatments to reduce the high prevalence of this complication..

However, type 2 diabetic patients often have peripheral neuropathy at diagnosis when preventative strategies will be too late (Young M et al. 1993a). Therefore, it is equally as important to understand the effects of peripheral neuropathy upon the diabetic foot in order to reduce the current toll of foot ulceration and amputations in patients with established neuropathy. A 50% reduction in amputations in diabetic patients is one of the stated aims of the St. Vincent Declaration (WHO/IDF 1988), and this has been shown to be achievable in specialised foot clinics (Edmonds et al. 1986, Thomson et al. 1991). In order to continue this improvement in the outlook for the diabetic foot, the patterns of foot abnormalities caused by peripheral neuropathy require further study.

Foot ulceration

Peripheral neuropathy is a major component of over 90% of diabetic foot ulceration (Edmonds et al. 1986, Thomson et al. 1991). The prevalence of foot ulceration and amputations in almost 6500 diabetic patients attending United Kingdom hospital diabetic clinics was reported to be 4.5% (Macleod et al. 1991), around 13% of the prevalence (28.7%) of peripheral neuropathy in the same sample (Young M et al. 1993a). A community based survey of type 2 diabetic patients registered with general practices in three cities in the North of England reported similar rates of peripheral neuropathy and foot ulceration (Kumar et al. 1992).

The principal mechanisms of foot ulceration in neuropathic patients are largely understood at the macroscopic level. Pressure, whether from walking or from inappropriate shoes, leads to autolysis of the skin and eventual breakdown if not removed (Koziak 1959, Brand 1983). The generation of higher plantar pressures during walking that has been reported in neuropathic patients (Veves et al. 1991b) is believed to be a function of the foot deformity, limited joint mobility and prominent metatarsal heads that are more common in these patients (Boulton et al. 1993). High dynamic plantar foot pressures, in patients with peripheral neuropathy (Masson et al. 1989b), have been shown to be predictive of foot ulceration (Veves et al. 1992b).

Neuropathic diabetic patients tolerate tight shoes. They also tolerate the initial inflammatory injury response to walking on high pressure areas (Brand 1983, MacFarlane et al. 1993). This is because neuropathic patients lack the normal protective sensation of pain which would lead to the removal of the shoes, or a change in gait to shift the abnormal loading.

At the microscopic level, it is believed that pressure overcomes the nutritive flow of the skin and this leads to localised tissue necrosis and breakdown (Flynn and Tooke 1992). The demonstration of increased arterio-venous shunting (Partsch 1978,

Boulton et al. 1982, Edmonds et al. 1982) in the diabetic foot, and the impaired hyperaemic injury response (Rayman et al. 1986, Walmsley et al. 1989) in neuropathic patients may also contribute to the increased risk of ulceration.

Bone changes in the diabetic foot

It has been reported that both type 1 and type 2 diabetic patients have a decreased bone mass when compared to age and sex matched control subjects (Selby 1988). Although renal failure secondary to diabetic nephropathy may contribute to this problem (Coburn 1980), there are suggestions that bone mass is reduced in diabetic patients with normal renal function. Edmonds et al. (1985) have shown that radioisotope uptake is increased in the feet of neuropathic diabetic patients. They suggested that this implied increased bone blood flow, as a consequence of increased arterio-venous shunting within bone, and increased osteoclastic activity. This increased osteoclastic activity may be part of the explanation for the reduced bone mass, and also has implications for the pathogenesis of Charcot neuroarthropathy, as outlined below.

Bone mass is measured by two methods; single photon absorptiometry (SPA) and dual energy X-ray absorptiometry (DEXA). Single photon absorptiometry was first described in 1963 (Cameron J et al.). The technique involves the measurement of the absorption of photons passed through the forearm at the proximal and distal radius. The forearm is placed in a water bath to provide a constant thickness of 'soft tissue' density, and the absorption of photons is then compared to a sample of bone with known mineral content. Dual energy X-ray absorptiometry (Stein et al. 1988) scans the entire skeleton and associated soft tissue with an X-ray beam of around 2mm diameter, the differential absorption of two energy intensities being used to derive the mineral content in a given area of bone. Reference ranges exist for the bone mass in the radius (using SPA), lumbar vertebrae and neck of femur (using DEXA) because of the increased risk of osteoporotic fractures in these regions, but are not widely reported for the feet (Seeman and Martin 1989).

The reduced bone mass reported in the feet of neuropathic diabetic patients (Cundy et al. 1985) can be expected to lead to a direct reduction in bone strength, as over 90% of the variance in bone strength is due to differences in bone mass (Carter and Hayes 1977). Such a reduction in bone strength, together with the increased risks of tripping and falling (Cavanagh et al. 1992), and the increase in repetitive trauma from higher foot pressures in neuropathic diabetic patients (Veves et al. 1991b) might be expected to contribute to the increased risk of foot fractures reported in diabetic patients (Paganini-Hill et al. 1981). Whilst the association between unperceived recurrent minor trauma and stress fractures has been made (Baldwin and Black 1986), the report of fractures with no history of injury (Coventry and Rothacker 1979) is probably a reflection of the degree of insensitivity to pain that may be present in neuropathic patients.

The risk of a fracture acting as an initiating event in Charcot neuroarthropathy has also been previously described (Johnson 1967, Connolly and Jacobsen 1985) and small periarticular fractures are a common early finding in patients with Charcot neuroarthropathy (McEnery et al. 1993).

Periosteal reaction along the metatarsal shafts is reported to be a common non-specific finding in radiographs of the diabetic foot (Williams et al. 1988), however another possible explanation for this finding is the elevated plantar pressure at the metatarsal heads in diabetic patients. In animal studies increased mechanical stress has been demonstrated to induce periosteal reactions (Uthoff and Jaworski 1985) but this association has not been made in man.

Other radiographic findings, including exostoses and pencilling of the metatarsal heads are reported as 'common' in diabetes (Geoffroy et al. 1979) but there are few studies with matched control groups to allow for valid comparisons of the true effects of diabetic neuropathy on the structure of the foot.

Medial arterial calcification

Medial arterial calcification has been reported to be more frequent in patients with diabetes than in healthy subjects (Morrison and Bogan 1929, Strandness et al. 1964, Ferrier 1964, Neubauer 1971), and is reported to be associated with diabetic peripheral somatosensory and autonomic neuropathy (Edmonds et al. 1982b, Goebel and Fuessi 1983, Everhart et al. 1988). Few previous studies have examined the distribution of medial arterial calcification quantitatively within the diabetic foot.

Medial arterial calcification is significantly associated with an increased prevalence of cardiovascular mortality (Everhart et al. 1988, Lachman et al. 1977, Nillson et al. 1967, Janka et al. 1980), although this may also be related to the increase in medial arterial calcification associated with diabetic nephropathy (Edmonds et al. 1982b, Goebel and Fuessi 1983), an independent marker of increased mortality in diabetes (Jensen et al. 1987).

Peripheral vascular disease is more common in diabetic patients than healthy control subjects (Janka et al. 1980) but the relationship between medial arterial calcification and the development of clinically important peripheral vascular disease is disputed (Neubauer et al. 1971, Goebel and Fuessi 1983, Nillson et al. 1967, Chanteleau et al. 1990). However, screening for the presence of peripheral vascular disease in diabetic patients usually involves clinical examination of the foot pulses and a measurement of the ankle brachial pressure index (Carter S 1969, Yao et al. 1969, Cutajar et al. 1973). Medial arterial calcification, when present, is known to alter the pulse waveform and falsely elevate ankle pressures in diabetic patients (Reimann and Bollinger 1974, European Consensus 1989). For this reason it has been suggested that toe systolic pressure measurements might replace ankle-pressure measurements as an index of arterial inflow to the diabetic foot (European Consensus 1989). However, the accuracy of toe pressure measurements would only be superior if medial arterial

calcification in the diabetic foot was less prevalent in the distal foot than the hind foot or ankle region and this has not been described in the literature and therefore such a study is required.

Charcot neuroarthropathy

The original text of Jean Marie Charcot's descriptions of a destructive arthropathy which starts 'rather suddenly, without precipitating cause' in neuropathic patients was recently translated into English and republished (Charcot 1992). The original French text was published in 1868 (Charcot 1868) and described Charcot's observations of patients with tabes dorsalis. However, it was not until 1936 that the first case report of neuroarthropathy in a diabetic patient was described (Jordan 1936). Since that time the devastating effects of Charcot neuroarthropathy in the diabetic foot have been acknowledged (Sinha et al. 1972, Cofield et al. 1983, Sammarco 1991), and diabetes is now the commonest cause of Charcot neuroarthropathy (Fryckberg 1987), but it is firmly held by those who regularly treat patients with Charcot feet that such changes are 'frequently overlooked' (Sanders and Frykberg 1993). The prevalence of Charcot change amongst 68,000 diabetic patients attending the Joslin Clinic was estimated as 0.15% (Sinha et al. 1972) but rates as high as 7% (of 242 patients) in other clinic populations (Pogonowska et al. 1967), and 2.9% in a selected group of 333 neuropathic diabetic patients (Cofield et al. 1983) have also been reported.

Eighty percent of the patients who develop Charcot neuroarthropathy have a known duration of diabetes of over 10 years (Sanders and Frykberg 1993). The duration of diabetes appears to be more important than age alone, as there is a report of a series of 18 type 1 diabetic patients with Charcot neuroarthropathy, in whom the average age was 33.5 years (range 25-52), but in whom the mean diabetes duration was 20 years (Clohisy and Thompson 1988). The long duration of diabetes prior to the initiation of the Charcot process reflects the degree of neuropathy that is usually present in these patients. Recently it has been suggested that a specific small fibre neuropathy of cool perception is present in diabetic patients with Charcot neuroarthropathy (Stevens M et al. 1992b) although this would seem unlikely given

the wide range of conditions, including leprosy and syphilis, in which Charcot neuroarthropathy occurs, and the density of neuropathy at the time of the initiating event.

The initiating event of the Charcot process is often a seemingly trivial injury (Sanders and Frykberg 1993), which may result in a minor periarticular fracture (McEnery et al. 1993) or in a major fracture (Johnson 1967, Connolly and Jacobsen 1985), despite the inability of the patient to recall the injury in many cases. Following this there is a rapid onset of swelling, an increase in temperature in the foot and often an ache or discomfort. The blood supply to the Charcot foot is always good (Sinha et al. 1972), indeed there are case reports of the Charcot process starting in patients following arterial bypass surgery (Edelman et al. 1987). It is assumed that autonomic neuropathy plays a part in the increased vascularity of bone, possibly by increased arterio-venous shunting (Edmonds et al. 1985), and this increases osteoclastic activity, resulting in the destruction, fragmentation, and remodelling of bone. It is these processes which, if left untreated, lead to the characteristic patterns of deformity in the Charcot foot (Sanders and Frykberg 1993), including the collapse of the longitudinal and transverse arches leading to a rocker bottom foot.

The natural history of Charcot neuroarthropathy passes from this acute phase of development through a stage of coalescence, in which the bone fragments are reabsorbed, the oedema lessens and the foot cools, into the stage of reconstruction, in which the final repair and regenerative modelling of bone takes place, to leave a stable, chronic Charcot foot (Eichenholtz 1966). The time course of these events is variable but intervention must be made in the earliest phase to prevent subsequent deformity.

The first principles of management are rest and freedom from weight-bearing. In the United States of America in particular, the practice of prolonged, (one year or more), immobilisation in a plaster of paris cast is the usual treatment (Sanders and Frykberg 1993). Even with this protracted period of casting the process can restart

once the cast is removed. The vogue for surgical stabilisation in the acute phase has now largely been abandoned because it often accelerated the destruction. However surgery may still be used, for example to remove a plantar prominence once the process has finally settled. The progress of the Charcot process may be determined by following clinical signs, skin temperature and radiographic change until they settle (Sanders and Frykberg 1991). In the United Kingdom total contact casting (Coleman et al. 1986), or bed-rest, are still the mainstays of treatment, although the Scotch-Cast Boot (Burden et al. 1983) is also used to provide a safer system of pressure redistribution. However, as yet there is no definitive treatment aimed at the underlying over-activity of osteoclasts in the active phase of Charcot neuroarthropathy.

Reflex sympathetic dystrophy (Sudeck's atrophy) is thought to be a similar process to Charcot neuroarthropathy, precipitated by an injury and associated with swelling, increased skin temperature, pain, and bone destruction (Duncan 1990). A clinical trial of the use of intravenous pamidronate (Aredia, Ciba-Geigy), in patients with reflex sympathetic dystrophy proved successful in reducing the amount of bone loss, the swelling and associated discomfort (Rehman et al. 1992), all of which are seen in acute Charcot neuroarthropathy. Pamidronate is a bisphosphonate, one of a relatively new class of therapeutic agents. Bisphosphonates are analogues of the naturally occurring compound pyrophosphate that become attached to the surface of hydroxyapatite crystals in the skeleton and are potent inhibitors of osteoclastic bone resorption. Because of this they have become widely used in the management of metabolic bone disease being particularly successful in the treatment of hypercalcaemia, osteoporosis and Paget's disease. The bisphosphonates are well tolerated and appear to have few long term side effects (Fleisch 1991). The success of pamidronate in the treatment of reflex sympathetic dystrophy led to the suggestion that this drug might prove useful in acute Charcot neuroarthropathy and to the pilot study described in Chapter 8.

Summary

Peripheral neuropathy is a factor in 90% of foot ulceration in diabetic patients and is believed to be associated with the presence of medial arterial calcification, which may be a marker of increased mortality in diabetic patients. If present to a significant degree in the ankle plexus of patients with diabetes then medial arterial calcification may interfere with ankle systolic pressure measurements. In addition, skeletal changes as a result of peripheral neuropathy, together with the increased risk of injury, might be expected to increase the risk of traumatic foot fractures in neuropathic patients. As traumatic fractures are believed to initiate Charcot neuroarthropathy, then this too may be more prevalent in neuropathic diabetic patients than previously assumed. However, controlled studies of radiographic changes in the feet of diabetic patients have not been reported to confirm these proposals.

The proposal of a specific neuropathic deficit in diabetic patients with Charcot neuroarthropathy seems unlikely given the broad range of conditions in which Charcot joints develop. If a generalised deficit was the usual pattern of neuropathy in diabetic patients with Charcot neuroarthropathy then this too could partly account for the high prevalence of Charcot change reported in some studies.

Even if Charcot neuroarthropathy is a rare complication of diabetic neuropathy, a definitive treatment is still required, and there is indirect evidence to suggest that intravenous pamidronate may prove successful in this condition.

Chapter Two

General Methods

General

All the studies performed in this thesis were approved by the Central Manchester Hospitals NHS Trust Ethical Committee. The patients and control subjects gave informed consent prior to their participation.

The microvascular and neurophysiological measurements described in this thesis were made with the subject resting semi-recumbent on a couch in a warm room, with the temperature maintained above 25 °C.

All the studies of rheological, fibrinolytic and microvascular parameters in diabetes were performed in the morning between 0900 and 1100. Patients and controls were asked to refrain from drinking tea, coffee or caffeine containing soft drinks from the evening prior to testing. Subjects were asked to attend after a light breakfast of orange juice and dry toast. Blood glucose was monitored during all the neurophysiological and haematological tests. No patient was hypoglycaemic during the tests. Hypoglycaemia, reported in the 24 hours prior to testing, led to a postponement of tests in two patients, who were tested later.

In all the studies HbA1c and HbA1 were measured in the routine biochemistry service of the Manchester Royal Infirmary. The assay method used, and result reported, by the laboratory was changed during the course of this thesis and therefore both HbA1c and HbA1 are used in this thesis. Where reported, insulin assays were measured independently by Dr Chris Gordon using a radioimmuno-assay for total insulin concentration with a total coefficient of variation of less than 9%.

Neurological assessments

Peroneal nerve motor conduction velocity

Peroneal nerve motor conduction velocity (MCV) was measured with standard surface electrodes. A Medelec (Medelec, Woking, UK) neurophysiology system was used for all recordings.

Nerve stimulation was performed proximally at the head of the fibula and distally on the shin, 10-15 cm above the ankle, with the recording electrode over extensor digitorum brevis on the lateral border of the foot. All stimulations were performed using a supramaximal stimulus. Averaging of 30 stimulations was used in severely neuropathic patients when the action potential was reduced. Tracings were taken of the motor action potentials and the latencies were measured by an independent observer without knowledge of the subject's identity or oxygen measurement.

The mean conduction velocity of both lower limbs was used for between group comparisons and in all correlation analyses, except where stated. In the studies of rheology and fibrinolysis in diabetic patients, patients were designated as neuropathic if they had a mean peroneal nerve motor conduction velocity of $<40 \text{ ms}^{-1}$ (Gregerson 1967, Young R et al. 1986, Ford et al. 1992).

In all intra-individual comparisons, the value for each leg was used as appropriate, as for example in the studies of the effects of arterial bypass surgery on peripheral nerve function, or in the study of neurological changes in Charcot patients, where the leg was designated as bypass or control, or Charcot or non-Charcot respectively.

Quantitative Sensory Tests

Vibration Perception Threshold

Vibration perception threshold was assessed using a Biothesiometer (Biomedical, Newbury, Ohio). The biothesiometers used during the course of the studies performed in this thesis had been recently calibrated, and where possible the same biothesiometer was used (Masson and Boulton 1990b).

In the studies of radiographic changes in diabetic patients (Section 2) the vibration perception threshold was expressed as the mean of five ascending thresholds from each foot with the probe balanced perpendicular under its own weight, measured at the hallux, first metatarsal head, fifth metatarsal head, heel and on the dorsum of the mid-foot. This method was chosen to give a wider spread of values around the biothesiometer measurement ceiling limit of 50 V, in order to reduce the number of subjects with unmeasurable vibration perception thresholds. A mean vibration perception threshold of 30 V was used as the dividing line between neuropathic and non-neuropathic groups because of the high median age of the subjects (Bloom et al. 1984).

In the study of the differences between Charcot and non-Charcot patients, vibration perception threshold was measured as the mean of three ascending thresholds measured with the probe balanced perpendicular, under its own weight, on the pulp of the great toe.

Thermal Perception Thresholds

Thermal perception thresholds were measured using the Amsterdam Thermoaesthesiometer and a forced choice protocol (Bertlesmann et al. 1985). The temperature of the skin of the chosen test site on the dorsum of the foot was measured using the integral thermo-couple, this was the reference temperature. One of the two test plates was set at the reference temperature and the second plate at a chosen temperature difference above or below the reference temperature. Each plate was then applied in turn, at random, and the subject asked which plate was warmer, whilst measuring warm thermal perception threshold, or cooler whilst measuring cool thermal perception threshold. The temperature difference was reduced until the subject was unable to accurately detect the difference in temperature. Repeated assessments with small variations around the presumed threshold are then performed, as in the method of limits. This determines the thermal perception threshold for an individual patient.

Neurological disability score

The neurological disability score used in the studies of Charcot and non-Charcot patients was that devised for the epidemiological survey of neuropathy in the United Kingdom, as this scoring system was known to correlate with vibration perception threshold, $r=0.8$, $p<0.001$ (Young M et al. 1993a). The score was derived from examination of the ankle reflexes, and vibration, pin-prick and temperature (cold tuning fork) sensation at the great toe in both feet. Each of the sensory modalities were scored as either normal=0 or abnormal=1 and reflexes as normal=0, present with reinforcement=1 or absent=2 for each side. Thus the maximum total deficit score was 10.

Autonomic function tests

Cardiovascular autonomic function tests were performed on patients in the study of differences between neuropathic patients with and without Charcot autonomic neuropathy. Three tests of heart rate variation were used to assess autonomic function. (Young R. et al 1986). These were:

1. R-R Inspiration : Expiration ratio during deep breathing

Patients were asked to inhale and exhale deeply for five breaths and the mean variation in R-R between inspiration and expiration was derived.

2. Valsalva manoeuvre

Patients were asked to perform a maximum expiration against a closed glottis for 15 seconds. The 30:15 ratio of R-R intervals before the manoeuvre and after release was then calculated.

3. Postural change

The lying:standing R-R interval ratio was also assessed.

All heart rate changes were compared to age related normal values (O'Brien et al. 1986), and were reported as abnormal if two of three were abnormal.

Vascular assessments

Transcutaneous oxygen measurements

Transcutaneous oxygen was measured using a TINA TCM3 meter, (Radiometer, Copenhagen, Denmark), at 44 °C on the dorsum of the foot over the muscle extensor digitorum brevis (EDB) in all studies. The electrode was calibrated with a standard oxygen / 20% carbon dioxide source and adjusted to atmospheric barometric pressure each time it was used. The electrode was attached to a self-adhesive fixation ring with a glycerol surface wetting agent added and left in place for 20 minutes. A stable reading of more than one minute after this time was used for the analysis.

The within subject coefficient of variation of the oxygen tension using this method was assessed by measuring one subject daily for ten days and was 2.4%. This is better than previously reported (Lukkari-Rautiainen et al. 1989), and may reflect the later model monitor used in this study, the improved calibration system, the longer site time allowed before a reading was taken, and the higher mean pO₂ of the subject tested.

Again, the mean transcutaneous oxygen of both feet was used in any correlation and regression models to limit the values to one per patient.

Laser Doppler flowmetry

Laser Doppler flowmetry was measured on the dorsum of the foot with a Periflux PF3 laser Doppler perfusion monitor (Perimed, Stockholm, Sweden) at native skin temperature before, and at 44 °C immediately following measurement of TcPO₂ and at the same site. Measurements were taken using the standard perpendicular probe and holder, attached to the skin with the double sided adhesive rings supplied. All readings were taken at 12 kHz bandwidth. The within subject coefficient of variation was measured at 17% at 44 °C. Readings were taken for 1 minute per recording.

Baseline value was set to zero.

Skin temperature

Skin temperature was measured using a Mikron Infra-Red thermometer (Mikron Instrument Company, Inc., New Jersey, USA).

In the studies of the relationships between tissue oxygenation, and reconstructive vascular surgery and neurological function (Chapter 4), the temperature of the foot was measured on the dorsum of each foot over the belly of EDB, after 30 minutes equilibration with room temperature. A mean of three readings was taken at each site. The within subject variation for this method was 3%.

In the study of the use of pamidronate in the treatment of Charcot neuroarthropathy (Chapter 9), to allow for the changes in temperature which might result from differences in ambient temperature over such a prolonged period of study, the temperature of the affected foot was expressed as the difference between the maximum temperature recorded on the affected side and the mean of four readings (three dorsal and one plantar) from the non-affected foot. The intra-individual coefficient of variation using this method was assessed by repeated measurement of patients during the assessment period prior to pamidronate treatment and was <3%.

Ankle Systolic Pressure and Ankle / Brachial Pressure Indices

Ankle systolic pressures were measured with a Doppler ultrasound stethoscope (Sonicaid, Oxford, UK). A standard blood pressure cuff was placed around the calf, above the ankle. The ultrasound probe was placed over the dorsalis pedis artery, or tibialis posterior if the dorsalis pedis was not detectable, and the cuff inflated until the flow was occluded. The ankle systolic pressure is taken as the pressure at which the pulse waveform returns. Incompressible arteries were given a pressure of 300mmHg.

The ankle brachial pressure index is derived by dividing the ankle systolic pressure by the brachial systolic pressure.

The mean ankle pressure and ankle brachial systolic ratios were used in all correlations to supply one value per patient in order to prevent the artificial elevation of the significance of the result which occurs when a given correlation has twice as many variables associated with it.

The Measurement of Rheological and Fibrinolytic Parameters

Rheological Parameters

General

All subjects had blood samples taken after resting semi-supine in a warm room, minimum temperature 24 °C, for at least ten minutes. Blood samples were drawn without occlusion using a 19G butterfly cannula into multiple polypropylene syringes and anticoagulated with potassium EDTA. All the blood samples in this study were analysed blindly by Mrs S Litherth and Mrs J Bennett to avoid potential bias of the results. Rheology was performed on whole blood (except plasma viscosity).

No measurement had a total coefficient of variation of greater than 10%.

Individual parameters

The following rheological parameters were assessed using recognised techniques (MacRury et al. 1991, 1993a,b).

Red cell deformability was measured using a Carri-Med St. George filtrometer (Carri-Med, Dorking UK) which measures the transit of red cells through a micropore filter (Dormandy et al. 1980). The results are expressed as red cell filtration index.

Red cell aggregation was measured using a photometric method with a Myrenne cone-plate aggregometer (Myrenne GMBH, Roetgen, Germany).

Plasma viscosity was measured at 37 °C using a Coulter-Harkness Viscometer (Coulter Electronics Ltd., Harpenden, Hertfordshire, UK).

Fibrinogen was measured using the Claus method (Claus 1957) in an ACL 300 Coagulation Laboratory analyser (Instrumentation Laboratory Ltd, Warrington UK.)

Whole blood viscosity

Whole blood viscosity was measured at 37 °C using a Contraves Low Shear 40 viscometer (Contraves Industrial products, Ruislip, UK). This uses a rotational bob viscometer method (Isogai et al. 1984), and is able to measure whole blood viscosity over a range of shear rates. The shear rates chosen in this study were 100 s⁻¹ (High Shear), 1 s⁻¹ (Low Shear) and 0.01 s⁻¹ (Very Low Shear), (MacRury and Lowe 1990, MacRury et al. 1991). The sample was measured at native haematocrit (measured in a Hawkesley microcentrifuge, Gallenkamp, Glasgow, UK) and then the result was corrected to a standard haematocrit of 45% as previously described (Matrai et al. 1987).

Measurement of Fibrinolytic Parameters

General

Blood samples were taken from the antecubital fossa with a 19G butterfly needle into multiple syringes. Samples were then mixed in a 0.9:0.1 ml ratio with sodium citrate in tubes which were kept at 4 °C on ice. Nothing was added to the samples for PAI estimation, but an additional step was required for the samples to be used in the t-PA activity assay. The citrated blood samples, in 1ml aliquots, were immediately acidified in 1ml acetate buffer solution. Both sets of samples were then centrifuged in a cooled centrifuge (temperature 4 °C) at 3000 rpm prior to being snap frozen in liquid nitrogen and stored at -70 °C until analysed simultaneously at the end of the study by Mrs S Liderth and Dr J Douglas, again without knowledge of an individual subject's study group.

Samples were measured before, and at the end of fifteen minutes venous occlusion of the arm. Venous occlusion is known to stimulate fibrinolysis in normal subjects (Wieczorek et al. 1993). Venous occlusion was achieved by placing a sphygmomanometer cuff around the upper arm and inflating to half-way between systolic and diastolic blood pressures (Wieczorek et al. 1993).

Activity assays were chosen because of the problems associated with binding of t-PA antigen during activated thrombosis leading to potentially inaccurate results (Grant 1991).

Individual parameters

The following direct measures of fibrinolytic activity were used:

Tissue Plasminogen Activator (t-PA) Activity

t-PA activity was analysed using an ELISA technique and a standard commercial test kit, (COA-SET t-PA, Chromogenix AB, Molndal, Sweden).

Plasminogen Activator Inhibitor (PAI-1) Activity

PAI-1 activity was analysed using an ELISA technique and a standard commercial test kit, (COA-SET PAI, Chromogenix AB, Molndal, Sweden).

All analyses were performed using the recommended standard procedure as described in the product instructions. The plates of ELISA test cells were read automatically using a Dynatech MR7000 plate reader (Dynatech Laboratories, Billingham, Sussex). The inter-assay coefficients of variation were around 6% for both the t-PA and PAI-1 assays.

Results were expressed as levels of Activity units ml^{-1} and also as t-PA/PAI-1 ratios to give a measure of the balance of fibrinolytic activity (Grant 1991).

Measurement of Prostacyclin and Thromboxane

The blood samples used for these assays were taken at the same time as the other samples, and without occlusion. The samples were placed into tubes containing EDTA and 0.05 ml of 0.04M Indomethacin solution, to inhibit arachidonic acid metabolism.

Prostacyclin

Prostacyclin (PGI_2) is unstable in plasma, with a half life of around three minutes, and degrades via hydrolysis to 6-keto-prostaglandin F_1 . This can then be measured, and, as the reaction displays first order kinetics, is a direct measure of prostaglandin production (Cho and Allen 1978). 6-keto-prostaglandin F_1 was measured using a standard commercial ELISA kit, Biotrak 6-keto-prostaglandin F_1 enzymeimmunoassay (EIA) system (Amersham International plc, Amersham, UK).

Thromboxane

Thromboxane (TXB_2) is the stable degradation product of Thromboxane A_2 and was the compound measured in this study to reflect thromboxane synthesis in vivo. TXB_2 was measured with a standard commercial test kit, Biotrak Thromboxane B_2 enzymeimmunoassay (EIA) system (Amersham International plc, Amersham, UK).

The Thromboxane B_2 was estimated using the standard liquid phase technique outlined in the assay instructions.

Both assays were read using the Dynatech MR 7000 automated plate reader. The concentrations of 6-keto-prostaglandin- F_1 and TXB_2 are expressed as ng ml^{-1} .

The 6-keto-PGF $_1$ and TXB_2 assays were each performed simultaneously using one kit only. The intra-assay coefficients of variation (using three assays of the internal standards) were 6.2% and 5.8% respectively.

Radiographic Techniques and Analysis

Technique for Weight Bearing Radiographs of the Feet

Weight-bearing (standing) true antero-posterior (A-P) and lateral radiographs were taken of each foot using a standardised protocol and fine grain x-ray film and screen (Kodak Ektamat G 100 film and X-omatic cassette with X-omatic fine intensifying screens, Kodak plc, Hemel Hempstead, UK). All the radiographs were taken by a single radiographer. True lateral radiographs were taken with a horizontal beam centred 2cm above the fifth metatarsal head, the x-ray focus-film distance (FFD) was 100cm, exposure 63 kV and 25 mAs. A-P radiographs were taken with a 20° cranial tilt from the vertical at FFD 80cm, 63 kV and 20 mAs. Each foot was imaged separately with the beam centred on the second metatarsophalangeal joint. A plasticine wedge filter was used for the dorsi-plantar view to create a uniform exposure over the toes. There is little radiosensitive tissue, and no bone marrow, in the adult foot and the radiobiological equivalent dose from these radiographs was calculated at 100th that of a standard chest x-ray (Judith Adams, personal communication).

Interpretation of Radiographic Findings

A scheme for interpretation of the radiographs was devised in which the abnormalities in the soft tissue, tendons, joints, and bones shown in Table 2.1 were assessed in different regions (Table 2.2) of each foot and ankle. Abnormalities, such as fractures (Figure 2.1) or evidence of infection, were assessed as either present or absent. Periosteal reaction (Figure 2.2), medial arterial calcification (Figure 2.3, 2.4) and similar quantitative changes were graded as absent, moderate, or marked. All the radiographs were interpreted by the same consultant radiologist (Dr. Judith Adams) who was unaware as to whether the film was from patient or control. An abnormality was judged present in a given patient if observed in one or both feet.

The designation of a Charcot process (Figure 2.5) required the simultaneous presence of bone and joint destruction and fragmentation, together with hypertrophic periosteal reaction (Cofield et al. 1983). Any missing bone, including individual phalanges, due to surgical intervention was deemed an amputation. Medial arterial calcification was defined as parallel tramline calcification and, if present, was graded as mild or severe according to previously published criteria (Morrison et al. 1929).

Table 2.1. Categories of abnormalities assessed for each component of the foot.

Bones	Joints	Soft Tissues
Destruction	Dislocation	Arterial calcification
Fractures	Exostoses	
Fragmentation	Narrowing	
Infection	Sclerosis	
Osteopaenia	Subluxation	
Pencilling	Widening	
Periosteal reaction		
Waisting		

Table 2.2. Regions of the foot examined for each category of abnormality.

Bones	Joints	Soft Tissue
Phalanges	Inter-phalangeal	Toes
Metatarsals	Metatarsophalangeal	Metatarsal
Tarsus	Lisfranc's	Mid-foot
Calcaneus	Intertarsal	Hindfoot
Talus	Chopart's	Ankle
Tibia/Fibula	Subtalar	
	Talocrural	



Figure 2.1. Fractures through the shaft of the fifth metatarsal and bases of the 2nd, 3rd and 4th metatarsals. The patient gave no history of trauma that might have resulted in these fractures.



Figure 2.2. Moderate degree of periosteal reaction along the shafts of the 2nd and 3rd metatarsals.



Figure 2.3. Heavy medial arterial calcification between the 1st and 2nd metatarsal shafts showing classical tramline appearance. Patient had peripheral neuropathy and a raised serum creatinine.



Figure 2.4. Heavy medial arterial calcification of the posterior tibial and plantar arteries.



Figure 2.5. Charcot neuroarthropathy. Destruction and fragmentation of the midfoot with loss of bone architecture and dislocation, and subluxation of the midfoot joints.

Bone Mass Measurement

Bone mass was measured at the proximal and distal radius using single photon absorptiometry (SPA) and in the lumbar spine and both femoral necks using dual energy absorptiometry (DEXA), as part of the routine clinical service of the University Department of Radiology. Results were compared to the age, sex and weight related normal ranges for this department. Results are expressed as Z scores of deviance from the normal bone mass (a score of 0). A Z score of -1 or less is indicative of increased fracture risk and -2 or less reflects highly significantly reduced bone mineral content (Seeman and Martin 1989).

Protocol for treatment with Pamidronate

Each subject received intravenous infusions of pamidronate according to an established protocol for the treatment of Paget's disease of bone (Anderson et al. 1993). Treatment commenced with an infusion of 30mg pamidronate (Aredia, Ciba Laboratories, Horsham, W Sussex, UK) in 250ml 0.9% saline over two hours. This was followed at fortnightly intervals by five further infusions of 60mg pamidronate in 500ml saline over four hours.

If patients had complained of any mild febrile reaction following the previous dose, they were given paracetamol 1g prior to subsequent infusions and advised to take paracetamol at regular intervals for 24 hours.

Prior to the commencement of each infusion, and two weeks after the final infusion, the skin temperature was measured in the Charcot and non-Charcot foot using the infra-red thermometer, (detailed on page 60), and blood was taken for serum creatinine, calcium, phosphate and alkaline phosphatase.

Statistical Methods

All the descriptive and standard comparative statistics were analysed using Minitab Software (Minitab Inc., State College, Pa, USA). Paired results were analysed using Student's t-test or Wilcoxon rank sum tests. Multiple comparisons were made using analysis of variance or Kruskal Wallis tests with appropriate follow up tests to determine the site of apparent differences. Correlations were analysed with Spearman Rank correlations and tests of association with Chi-squared tests.

The complex statistical methods used to model the relationship of medial arterial calcification and neuropathy, and the analysis of the response to Pamidronate, are detailed in the appropriate sections of this thesis, in proximity to the results they describe, to aid cross reference.

Section Two

Studies of the Microvascular Hypothesis of the Aetiology of Diabetic Neuropathy

Chapter Three

Rheology, Fibrinolysis and Microvascular Blood Flow in Diabetic Patients and Non-Diabetic Control Subjects

Patients and Control Subjects

The diabetic patients were selected from the computer database of the Manchester Diabetes Centre. No diabetic patient had microalbuminuria, or retinopathy, as assessed by fundoscopy through dilated pupils. Microalbuminuria was assessed by timed overnight collection of urine, and no patient had an overnight albumin excretion rate of more than $20 \mu\text{g min}^{-1}$. Neuropathy was defined as a peroneal conduction velocity of less than 40 ms^{-1} . Twelve neuropathic type 1 diabetic patients were age and sex matched to twelve non-neuropathic type 1 patients (Table 3.1). Twelve type 2 neuropathic patients were similarly matched to twelve non-neuropathic patients (Table 3.2). For the purposes of these studies the type 1 and type 2 diabetic patients were considered in combined groups of neuropathic and non-neuropathic patients (Table 3.3).

Twenty-four control subjects were age and sex matched to the diabetic subject groups. Control subjects were drawn from the staff of the Manchester Royal Infirmary and spouses of the diabetic subjects, and all were in good health at the time of study. Control subjects were asked about symptoms of diabetes, and as to whether they had a family history of diabetes; no positive responses were obtained. All had a random blood glucose measured during the study, and all were less than 7 mmol l^{-1} .

No diabetic patient or control subject smoked, and none was taking aspirin, non-steroidal anti-inflammatory drugs or evening primrose oil at the time of study.

Patients taking vasoactive medication, such as β -blockers, calcium antagonists and ACE inhibitors were also excluded from the study.

Table 3.1. Physical characteristics of the type 1 patients in this study.

	Type 1 Non Neuropathic	Type 1 Neuropathic
Number	12	12
Age (years)	43.4 (33.3-47.5)	38.6 (35.1-46.4)
Duration of diabetes (years)	31.0 (15.0-33.0)	22.0 (11.2-27.7)
Insulin (U ml ⁻¹)	21.0 (14.0-28.0)	40.0 (5.0-110.0)
Glucose (mmol l ⁻¹)	7.4 (4.9-18.7)	16.4 (13.0-21.8)
HbA1 (%)	10.0 (7.8-11.8)	10.2 (8.9-11.3)
Total Cholesterol (mmol l ⁻¹)	5.20 (4.80-5.60)	5.8 (5.4-6.6)
Creatinine (μmol l ⁻¹)	81.0 (71.5-86.2)	79.0 (75.0-97.0)
Weight (kg)	70.0 (62.8-83.8)	71.3 (60.0-88.0)
Height (m)	1.73 (1.66-1.75)	1.72 (1.65-1.80)
BMI (kg m ⁻²)	23.4 (21.5-28.1)	22.9 (22.0-26.8)
Systolic blood pressure (mmHg)	116 (110-139)	126 (116-145)
Diastolic blood pressure (mmHg)	70 (61-80)	70 (63-80)

Results are shown as median (interquartile range).

There are no significant differences between any of the parameters.

Table 3.2. Physical characteristics of the type 2 patients in this study.

	Type 2 Non-Neuropathic	Type 2 Neuropathic
Number	12	12
Age (years)	49.2 (44.5-53.5)	56.5 (49.9-60.6)
Duration of diabetes (years)	5.0 (4.4-15.5)	4.0 (3.0-10.0)
Insulin (U ml ⁻¹)	11.5 (5.5-18.5)	9.0 (6.2-17.7)
Glucose (mmol l ⁻¹)	11.0 (8.7-13.6)	9.8 (8.7-12.7)
HbA1 (%)	8.5 (8.0-9.7)	8.8 (7.7-10.6)
Total Cholesterol (mmol l ⁻¹)	6.6 (5.3-6.9)	5.3 (4.8-7.5)
Creatinine (μmol l ⁻¹)	76.0 (64.2-81.2)	87.0 (67.0-92.0)
Weight (kg)	77.7 (69.6-86.7)	80.2 (70.0-87.9)
Height (m)	1.66 (1.62-1.72)	1.72 (1.67-1.76)
BMI (kg m ⁻²)	28.9 (69.6-86.7)	26.5 (23.9-31.6)
Systolic blood pressure (mmHg)	139 (116-159)	164 (143-173)
Diastolic blood pressure (mmHg)	82 (75-90)	84 (76-92)

Results are shown as median (interquartile range).

There are no significant differences between any of the parameters.

Table 3.3. Physical characteristics of the study groups

	Non-diabetic control subjects (C)	Non-neuropathic diabetic patients (D)	Neuropathic diabetic patients (N)
Number	24	24	24
Male:Female	11:13	13:11	11:13
Age (range) (years)	42.5 (25-68)	44.1 (29-60)	48.1 (26-67)
Type of diabetes I:II	-	12:12	12:12
Duration of diabetes (years)	-	17.4 ± 11.6	15.6 ± 12.3
Creatinine (μmol l ⁻¹)	63.0 ± 11.0	77.1 ± 10.8	85.1 ± 11.9*
Cholesterol (mmol l ⁻¹)	4.6 ± 0.7	5.5 ± 1.1	6.0 ± 0.9*
HbA1 (%)	-	9.3 ± 2.0	9.9 ± 1.8
Insulin (U ml ⁻¹)	9.6 ± 6.6	15.8 ± 8.8	18.5 ± 28.2
Glucose (mmol l ⁻¹)	5.0 ± 0.7	11.9 ± 6.9	14.9 ± 6.3
Systolic BP (mmHg)	120 ± 20	125 ± 23	145 ± 22*
Diastolic BP (mmHg)	74 ± 12	73 ± 11	78 ± 11
BMI (kg m ⁻²)	25 ± 3	22 ± 5	26 ± 5

All results (except age) are shown as mean ± SD. Age is shown as mean (range).

There were no significant differences between any of the parameters except creatinine (p<0.05 N vs. C), cholesterol (p<0.01 N vs C), and systolic blood pressure (p<0.05 N vs. C).

Methods

All diabetic patients and control subjects were seen after a light fat free breakfast. No diabetic subject was hypoglycaemic at the time of study, or had symptomatic or biochemical hypoglycaemia for 24 hours preceding the study. After resting semi-recumbent in a warm room for at least ten minutes, blood samples were drawn without occlusion for rheological and fibrinolytic parameters. Venous occlusion was then performed using a sphygmomanometer cuff inflated to half way between systolic and diastolic blood pressures for 15 minutes after which time blood was drawn for post venous occlusion assessments of fibrinolysis. The measurement of the rheological and fibrinolytic parameters is further described in the Methods (Chapter 2, pages 62-67).

Transcutaneous oxygen tensions and laser Doppler flux were then measured on the dorsum of the foot over extensor digitorum brevis. Finally, measurements of the peroneal nerve motor conduction velocities in both legs were performed. The microvascular and neurophysiological assessment methods are detailed in Chapter 2, pages 53-60.

Statistical Analyses

Multiple group comparisons were initially performed by analysis of variance and Kruskal-Wallis tests (Minitab Software, Minitab Inc., State College, Pa.), with appropriate follow up tests.

Results

Rheological Parameters

Patients

The type 2 diabetic patients were significantly older than the type 1 patients and had a shorter duration of diabetes (Tables 3.1, 3.2). However, as only red cell filtration correlated weakly with duration of diabetes $r = -0.35$, $p < 0.05$, and was not significantly different between the type 1 and type 2 diabetic patients, the two types of diabetes were combined to form one group of neuropathic patients and one group of non-neuropathic patients with equal numbers of type 1 and type 2 patients in each (Table 3.3).

Individual parameters

The results of the neurophysiological, circulatory and rheological parameters are shown in Table 3.4.

Nerve conduction was reduced in the 'non-neuropathic' diabetic patients when compared to control subjects and by definition was lower in the neuropathic diabetic patients ($p = 0.043$ C vs D, $p < 0.0001$ D vs N). Transcutaneous oxygen tension, measured at 44 °C, was significantly lower in the neuropathic diabetic patients when compared to non-diabetic control subjects and non-neuropathic diabetic patients ($p = 0.017$ N vs C and N vs D). There was no significant difference in the laser Doppler flux between the non-neuropathic diabetic patients and the non-diabetic control subjects, but the neuropathic diabetic patients had a lower laser Doppler flux when compared to controls (C 72 ± 40 vs D 64 ± 41 vs N 50 ± 26 flow units, $p = \text{NS}$ C vs D, $p = 0.04$ N vs C).

No significant change in any of the rheological parameters measured was found between diabetic patients without neuropathy and the non-diabetic control subjects. Red cell deformability was similar in all groups (Table 3.4).

The neuropathic diabetic patients had a significantly higher red cell aggregation, plasma viscosity, and fibrinogen concentration than non-diabetic control subjects (Table 3.4). Red cell aggregation and fibrinogen concentrations were higher in the neuropathic diabetic patients than in the non-neuropathic diabetic patients (Table 3.4).

Whole blood viscosity at 100 s^{-1} and 1 s^{-1} was significantly higher in the neuropathic diabetic patients compared to the non-diabetic control subjects. There were no significant differences in whole blood viscosity at these shear rates between neuropathic and non-neuropathic diabetic patients, and no significant differences between all groups measured at 0.01 s^{-1} (Table 3.4).

In the diabetic patients as a whole, fibrinogen concentrations correlated negatively with transcutaneous oxygen measurements $r = -0.49$, $p < 0.001$ and peroneal nerve conduction velocity $r = -0.40$, $p < 0.01$. Red cell aggregation was significantly negatively correlated with transcutaneous oxygen concentration $r = -0.36$, $p = 0.02$, but not with peroneal conduction velocity, $r = -0.27$ ($p = 0.1$).

Table 3.4. Results of microvascular and rheological tests in each of the three groups of subjects.

	Non-diabetic control subjects	Non- neuropathic diabetic patients	Neuropathic diabetic patients	p value
Peroneal conduction velocity (ms ⁻¹)	51.7 ± 6.0	45.1 ± 5.2	34.5 ± 5.8	p<0.05 C vs D p<0.01 N vs C
Transcutaneous oxygen (mmHg)	76.0 ± 16.3	71.1 ± 10.3	62.9 ± 8.7	p<0.05 N vs C
Laser Doppler flux (flow units)	72 ± 40	64 ± 41	50 ± 26	p<0.05 N vs C
Red cell filtration (U)	0.54 ± 0.03	0.52 ± 0.05	0.52 ± 0.04	p=NS
Red cell aggregation (U)	6.5 ± 1.8	6.7 ± 1.6	8.8 ± 2.0	p<0.05 N vs C
Plasma viscosity (mPas ⁻¹)	1.21 ± 0.04	1.24 ± 0.10	1.27 ± 0.06	p<0.05 N vs C
Fibrinogen (g l ⁻¹)	2.9 ± 0.6	3.0 ± 0.8	3.6 ± 0.8	p<0.05 N vs C
Whole blood viscosity (Corrected to 45% haematocrit) (mPas-1)				
100 s ⁻¹	5.2 ± 0.4	5.4 ± 0.8	5.7 ± 0.6	p<0.05 N vs C
1 s ⁻¹	17.5 ± 2.9	17.9 ± 3.7	19.6 ± 3.5	p<0.05 N vs C
0.01 s ⁻¹	67.4 ± 17.7	60.4 ± 12.2	61.5 ± 18.4	p=NS

All results are shown as mean ± SD.

Thromboxane and Prostacyclin

Thromboxane (TXB₂)

There was no significant difference in plasma TXB₂ concentrations between the diabetic groups and the non-diabetic control subjects. (Table 3.5).

Prostacyclin

Peripheral venous prostacyclin was reduced in neuropathic diabetic patients compared to non-diabetic control subjects, mean $0.11 \pm 0.07 \text{ ng ml}^{-1}$ vs $0.18 \pm 0.05 \text{ ng ml}^{-1}$, $p=0.03$, with non-neuropathic diabetic patients having an intermediate level of prostacyclin ($0.18 \pm 0.13 \text{ ng ml}^{-1}$), (Table 3.5).

Prostacyclin / Thromboxane ratio

The balance of Prostacyclin / Thromboxane concentrations was also reduced in neuropathic diabetic patients ($0.65 \pm 0.30 \text{ ng ml}^{-1}$) when compared to non-diabetic control subjects ($0.91 \pm 0.21 \text{ ng ml}^{-1}$), $p=0.01$, but just failed to reach standard significance versus non-neuropathic diabetic patients ($0.84 \pm 0.26 \text{ ng ml}^{-1}$), $p=0.07$.

Table 3.5. Thromboxane B₂ and 6-keto prostaglandin F₁ concentrations in peripheral venous blood in neuropathic (N) and non-neuropathic (NN) diabetic patients and non-diabetic controls (C).

	N	NN	C	p value
6-keto PGF ₁ (ng ml ⁻¹)	0.11 ± 0.07	0.18 ± 0.13	0.18 ± 0.06	$=0.03 \text{ N vs C}$
TXB ₂ (ng ml ⁻¹)	0.17 ± 0.08	0.18 ± 0.14	0.19 ± 0.05	NS
PC / TXB ₂ ratio	0.65 ± 0.30	0.84 ± 0.26	0.91 ± 0.21	$=0.01 \text{ N vs C}$

Results are expressed as mean \pm SD.

Fibrinolytic parameters.

Results

There was no difference in basal or stimulated fibrinolysis between type 1 and type 2 patients (Table 3.6). Therefore, as with rheological variables, the two types of diabetes are considered together as two groups, non-neuropathic and neuropathic patients, for comparison with non-diabetic controls. The results are detailed in table 3.7.

In summary, when comparing the non-neuropathic and neuropathic diabetic patients with non-diabetic controls, basal levels of t-PA activity were similar in all groups. In the diabetic groups however the activity of PAI-1, the principal inhibitor of t-PA, was markedly increased. This resulted in significantly lower t-PA/PAI-1 ratios in the diabetic groups. There was no significant difference between the neuropathic and non-neuropathic patients in basal levels of t-PA, PAI-1 or in the t-PA/PAI-1 ratio.

Following 15 mins venous occlusion, the stimulated fibrinolytic response was significant greater in control subjects than non-neuropathic diabetic patients (t-PA/PAI-1 ratio C 3.96 vs D 0.44, $p < 0.001$), rising 21 times baseline.

The final t-PA/PAI-1 ratios (t-PA/PAI-1 D 0.44 vs N 0.41, $p = 0.38$), and fibrinolytic response to venous occlusion were similar in neuropathic and non-neuropathic diabetic patients (D 8.5 vs N 5.0, $p = 0.67$).

Table 3.6. Results of basal and stimulated fibrinolytic parameters in type 1 and type 2 diabetic patients with no evidence of complications.

	Type 1 Diabetic Patients	Type 2 Diabetic Patients
Basal		
t-PA (Au ml ⁻¹)	1.0 (0.1-1.)	0.5 (0.1-1.4)
PAI-1 (Au ml ⁻¹)	12.5 (2.5-15.0)	11.5 (7.7-20.4)
t-PA/PAI-1 ratio	0.09 (0.02-0.17)	0.05 (0.01-0.13)
Post Venous Occlusion		
t-PA (Au ml ⁻¹)	3.5 (0.7-6.2)	5.2 (1.9-8.0)
PAI-1 (Au ml ⁻¹)	3.5 (0.1-16.7)	7.0 (0.8-20.2)
t-PA/PAI-1 ratio	1.42 (0.09-4.10)	0.46 (0.06-2.10)

Results are shown as median and interquartile range.

All comparisons p=NS.

Table 3.7. Results of basal and stimulated fibrinolytic parameters in control subjects, and non-neuropathic and neuropathic diabetic patients.

	Control subjects (C)	Non-Neuropathic (D)	Neuropathic (N)	p
Basal				
t-PA (Au ml ⁻¹)	0.9 (0.4-1.2)	0.9 (0.1-1.7)	0.4 (0.1-1.2)	*
PAI-1 (Au ml ⁻¹)	5.0 (3.4-8.3)	13.0 (5.5-15.5)	12.0 (2.0-19.5)	**
t-PA/PAI-1	0.16 (0.06-0.39)	0.06 (0.01-0.14)	0.08 (0.01-0.12)	**
Post Venous Occlusion				
t-PA (Au ml ⁻¹)	4.5 (3.6-6.8)	6.5 (2.2-7.4)	4.9 (1.7-7.6)	*
PAI-1 (Au ml ⁻¹)	1.0 (0.5-7.0)	4.5 (0.1-15.0)	7.0 (0.4-20.4)	*
t-PA/PAI-1	3.96 (0.50-8.89)	0.44 (0.01-1.44)	0.41 (0.11-3.23)	***

Results are shown as median and interquartile range.

* p=NS C vs D vs N.

** p<0.05 C vs D or N

*** p<0.05 C vs. N

Correlations

Fibrinolytic activity was weakly inversely correlated with body mass index in both diabetic patients and control subjects, and diastolic blood pressure in controls. PAI-1 levels increasing and t-PA levels generally decreasing with increasing BMI or blood pressure. Insulin and blood glucose concentration were not significantly correlated with fibrinolytic activity in diabetic patients or controls, nor was HbA1 in the diabetic groups.

Table 3.8. Spearman rank correlation coefficients for t-PA and PAI-1 with various indicators of 'insulin resistance' and diabetes control.

	Diabetic patients		Control subjects	
	PAI-1	t-PA	PAI-1	t-PA
BMI	0.36*	-0.21	0.26	-0.45*
Diastolic	0.12	-0.13	0.39*	-0.43*
Insulin	-0.18	-0.08	-0.14	-0.14
Glucose	0.08	-0.18	-0.26	0.05
HbA1	-0.17	0.08		

* $p<0.05$

Tissue oxygenation and nerve function

Results

Diabetic Patients

In the diabetic patients Peroneal Nerve MCV correlated with TcPO₂, $r=0.59$ $p<0.001$, (Figure 3.1), but not with skin temperature, $r=0.16$ $p=NS$.

Comparing the leg with the higher TcPO₂ (mean 70.2 ± 9.3 (SD) mmHg) with that with the lower TcPO₂ (61.7 ± 2.1 mmHg) in each patient, peroneal nerve MCV was significantly higher, 45.3 ± 7.1 vs 41.5 ± 6.3 ms⁻¹, $p<0.001$, though no significant difference in skin temperature was observed, 31.4 ± 0.4 vs 31.1 ± 0.5 °C.

Non-Diabetic Control Subjects

In the non-diabetic controls subjects a similar correlation was found between tissue oxygenation and nerve conduction, $r = 0.7$, $p<0.01$, Figure 3.2.

Figure 3.1. Relationship between mean transcutaneous oxygen and mean peroneal conduction velocity in diabetic patients.
 $r = 0.59, p < 0.001$

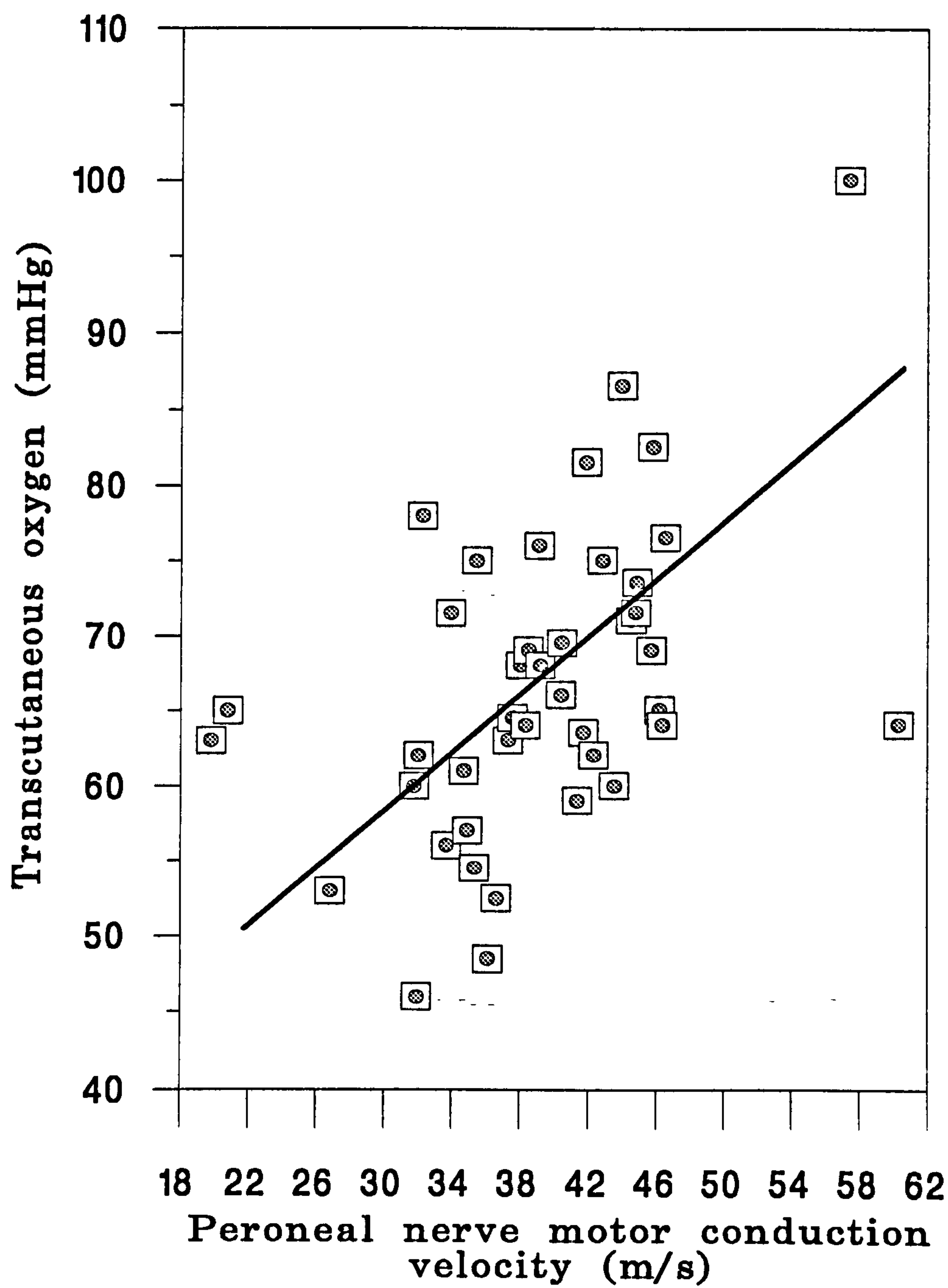
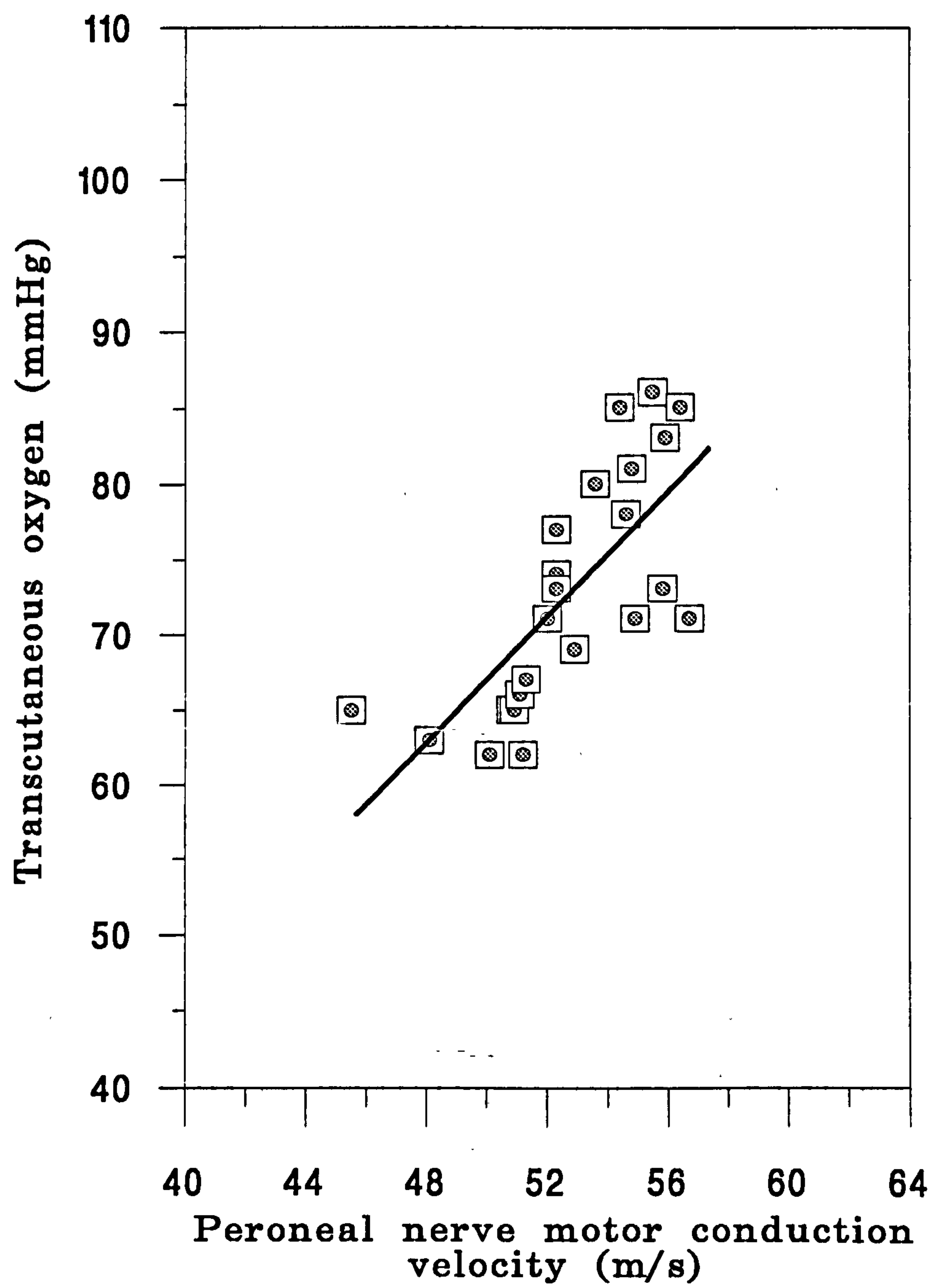


Figure 3.2. Relationship between transcutaneous oxygen and peroneal conduction velocity in non-diabetic controls. $r= 0.7$, $p<0.01$



Laser Doppler Flowmetry and Microvascular Reactivity

Results

The results are detailed in Table 3.9 and Figures 3.3-3.5.

At native skin temperature (N 30.6 ± 2.0 °C, D 30.4 ± 1.4 °C, C 29.3 ± 2.1 °C), there were no significant differences in laser Doppler flux between the three groups of subjects (Table 3.9). There were also no significant differences in skin temperature between the three groups.

Heating the skin to 44 °C caused a significant rise in laser Doppler flux in all three groups of patients (Figures 3.3-3.5). There was no significant difference in the maximal laser Doppler flux between neuropathic and non-neuropathic diabetic subjects. The mean maximal Laser doppler flux was 57.0 ± 38.4 perfusion units in the diabetic patients and Laser doppler flux correlated with TcPO₂, $r = 0.36$, $p < 0.01$ (Figure 3.6).

The laser Doppler flux at 44 °C was significantly lower in the neuropathic diabetic patients when compared to control subjects (median (range) 49.0 (33.5-60.5) vs 76.5 (47.5-84.7), $p = 0.029$).

These results were also reflected in the proportional increase in laser Doppler flux in response to heating the skin to 44 °C. The increase in laser Doppler flux in non-diabetic subjects was 75.0 (56.0-88.2) times baseline, compared to 25.0 (11.9-70.0) in non-neuropathic diabetic patients ($p = 0.03$ vs Controls) and 29.2 (5.6-51.4) times baseline in neuropathic subjects ($p = 0.005$ vs Controls).

There was no significant difference in the proportional increase in laser Doppler flux in neuropathic compared to non-neuropathic patients ($p=0.63$).

Table 3.9. Results of laser Doppler flux (LDF) measurements in the non-diabetic control subjects and the neuropathic and non-neuropathic diabetic patients.

	Non-diabetic control subjects	Non-neuropathic diabetic patients	Neuropathic diabetic patients
Basal			
Mean LDF	1.2 ± 0.4	4.8 ± 4.7	2.8 ± 3.1
Median LDF	1.0 (0.8-1.6)	3.5 (0.7-7.2)	1.4 (0.7-3.5)
44 °C			
Mean LDF	72.1 ± 38.7	64.2 ± 41.2	50.0 ± 22.3
Median LDF	76.5 (47.7-84.7)	42.0 (38.0-90.0)	49.0 (33.5-60.5)
Proportional Increase in LDF			
Mean	69.4 ± 23.9	49.1 ± 61.6	31.2 ± 24.7
Median	75.0 (56.0-88.2)	25.0 (11.9-70.0)	29.2 (5.6-51.4)

Results are shown as mean ± SD and median (interquartile range).

Figure 3.3. Laser Doppler flux at native skin temperature and at 44 C in neuropathic diabetic patients.

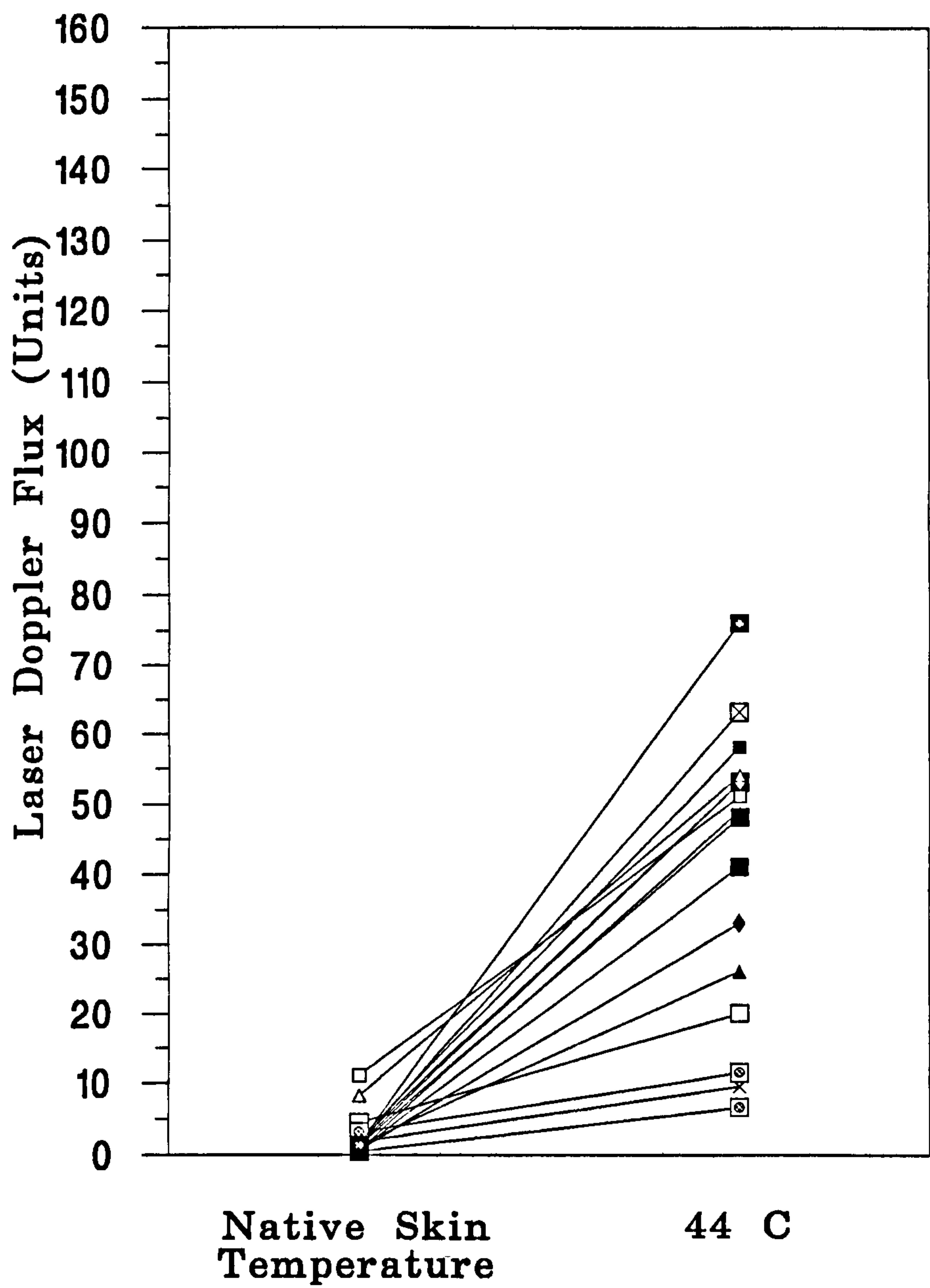


Figure 3.4. Laser Doppler flux at native skin temperature and at 44 C in non-neuropathic diabetic patients.

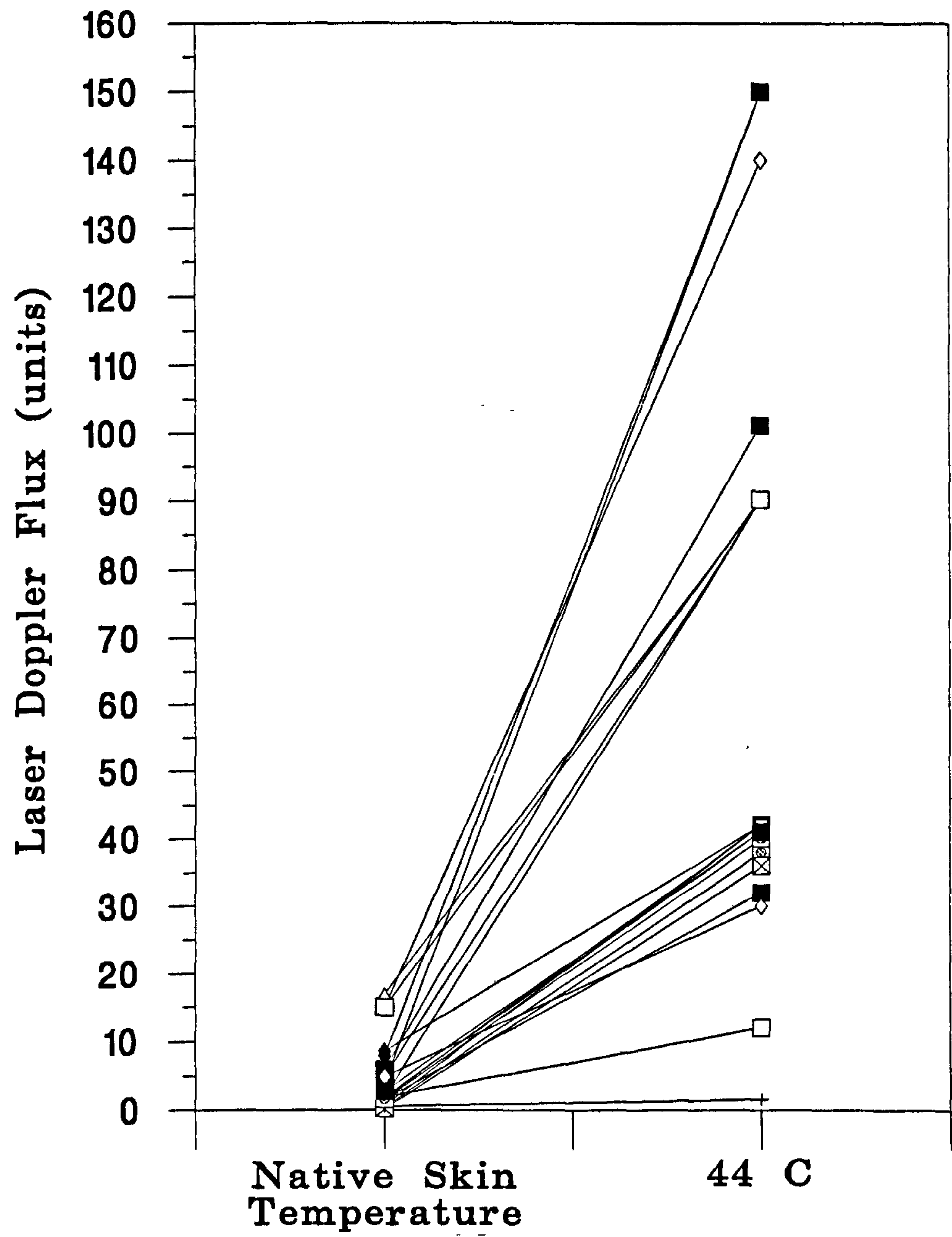


Figure 3.5. Laser Doppler flux at native skin temperature and at 44 C in non-diabetic controls.

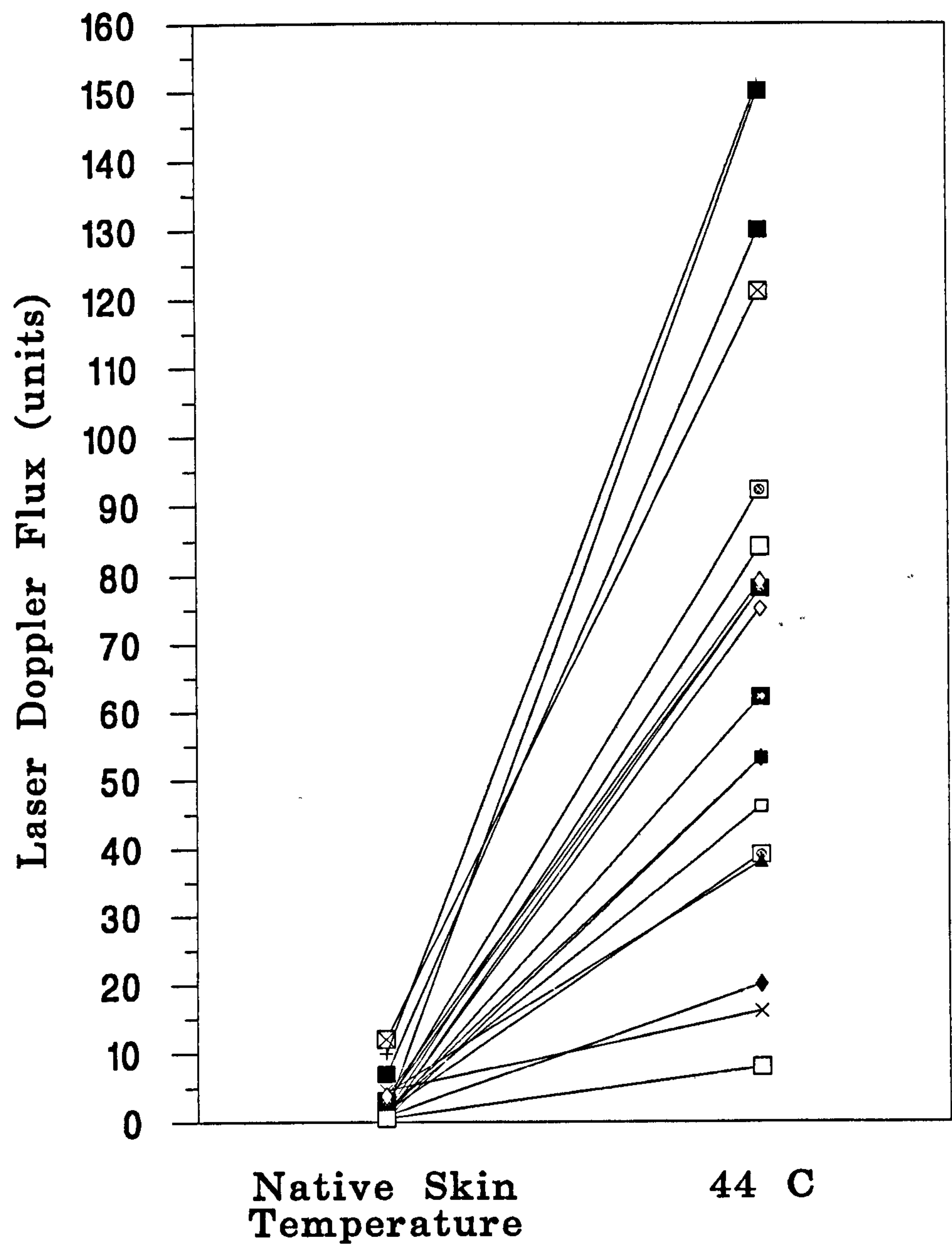
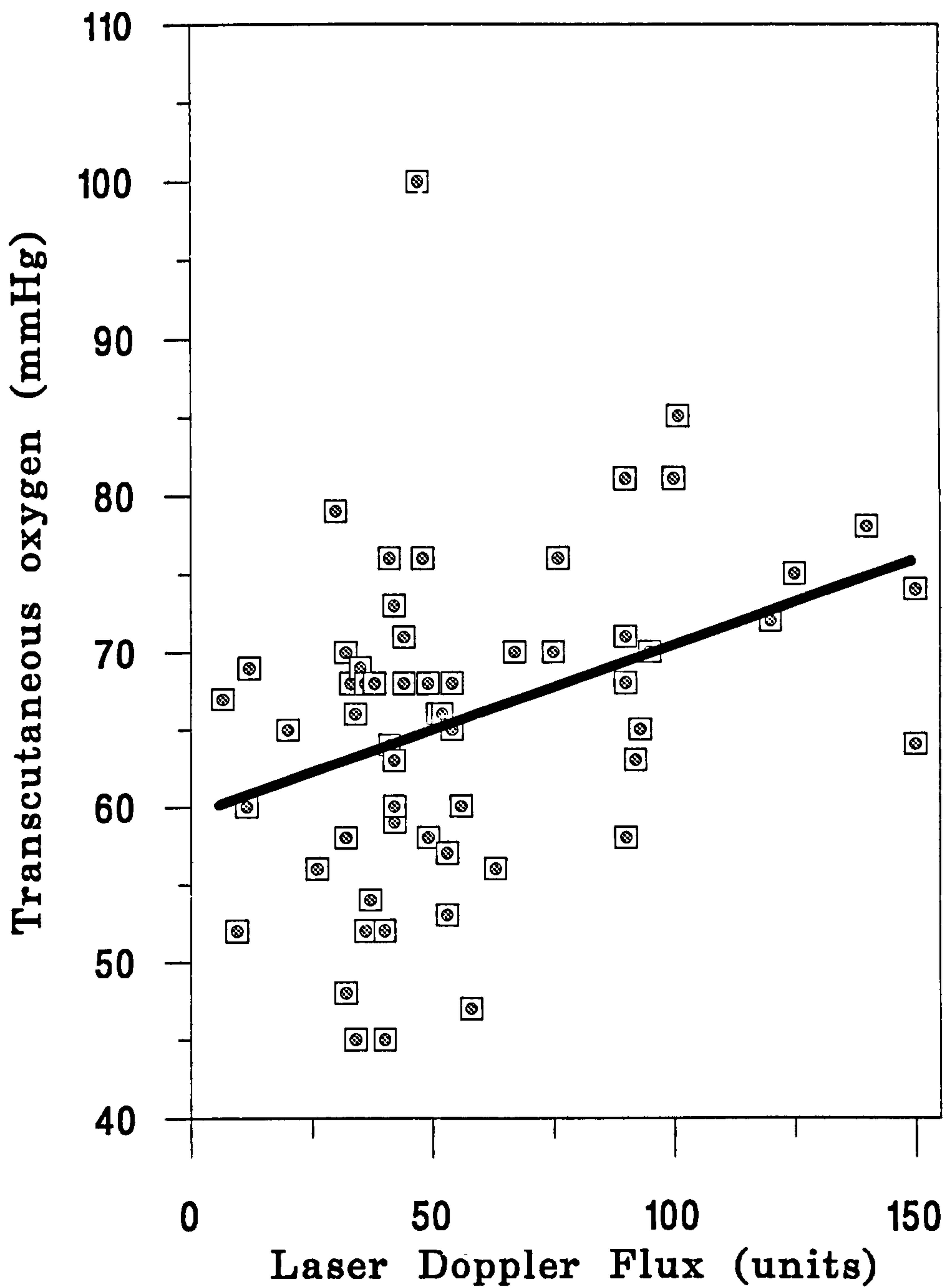


Figure 3.6. Graph of the relationship between laser Doppler flux at 44 C and transcutaneous oxygen tension in the diabetic patients. Spearman rank correlation $r= 0.36$, $p<0.01$.



Chapter Four

The Effects of Revascularisation on Peripheral Nerve Function

Patients and Methods

Non-Diabetic Patients

The non-diabetic patients selected for the study comprised ten consecutive patients with no past history of diabetes, mean age 59 (range 52-77) years, with angiographically proven superficial femoral artery thromboses suitable for unilateral femoro-popliteal bypass.

All non-diabetic patients had fasting plasma glucose measurements on the day of operation and all were less than 6 mmol/l.

Diabetic Patients

The diabetic patient group comprised six consecutive type 2 diabetic patients with superficial femoral thromboses, median age (interquartile range) 64 (58-72) years, duration of diabetes 7 (3-14) years.

All the patients were under the care of Mr. MG Walker, Consultant Vascular Surgeon at the Manchester Royal Infirmary, but were otherwise unselected.

Methods

All subjects had transcutaneous oxygen, peroneal nerve MCV and skin temperature measured in both legs, using the techniques described on pages 54, 59, and 60. The measurements were made the day before and six to eight weeks following surgery.

The contralateral leg, which did not undergo surgery, was used as the control in all cases.

Results

Non-Diabetic Patients

In the bypass leg TcPO₂ increased from 59.3 ± 10.7 mmHg preoperatively to 70.7 ± 7.2 mmHg postoperatively ($p < 0.01$) (Figure 4.1). Peroneal nerve MCV increased from 42.6 ± 6.1 to 46.7 ± 3.2 ms⁻¹ ($p < 0.01$) (Figure 4.2), but skin temperature was unchanged 30.3 ± 0.4 vs 30.4 ± 1.3 °C ($p = \text{NS}$).

There was no significant difference in TcPO₂ 63.2 ± 8.8 vs 63.0 ± 4.6 mmHg, peroneal nerve MCV 45.1 ± 7.8 vs 43.4 ± 7.2 ms⁻¹ or skin temperature 30.8 ± 1.3 vs 30.2 ± 1.2 °C (all $p = \text{NS}$) in the unoperated leg.

Diabetic Patients

In the revascularised legs, transcutaneous oxygen increased from a median 37.5 (28.5-45.7 interquartile range) mmHg to 55.5 (53.5-62.5), $p = 0.036$, mean increase 20.2 (14.8-25.6 95% confidence intervals) (Figure 4.3). This was accompanied by a significant improvement in peroneal nerve MCV from a median 31.7 (26.5-36.3) ms⁻¹ to 33.5 (32.9-39.4) ms⁻¹, $p = 0.04$. The mean increase in peroneal nerve MCV was 4.7 (1.7-7.7 95% CI) ms⁻¹ (Figure 4.4).

There was no significant change in transcutaneous oxygen or peroneal nerve MCV in the contralateral limb. The median transcutaneous oxygen tension was 58.0 (49.2-66.0) mmHg pre operation and 53.0 (45.0-65.5) mmHg post, and peroneal nerve MCV, pre 35.5 (25.5-39.5) ms⁻¹, post 34.2 (26.7-40.3) ms⁻¹, $p = 0.83$ and $p = 1.0$ respectively.

Figure 4.1. Graph showing improvement in transcutaneous oxygen in non-diabetic patients following revascularisation.

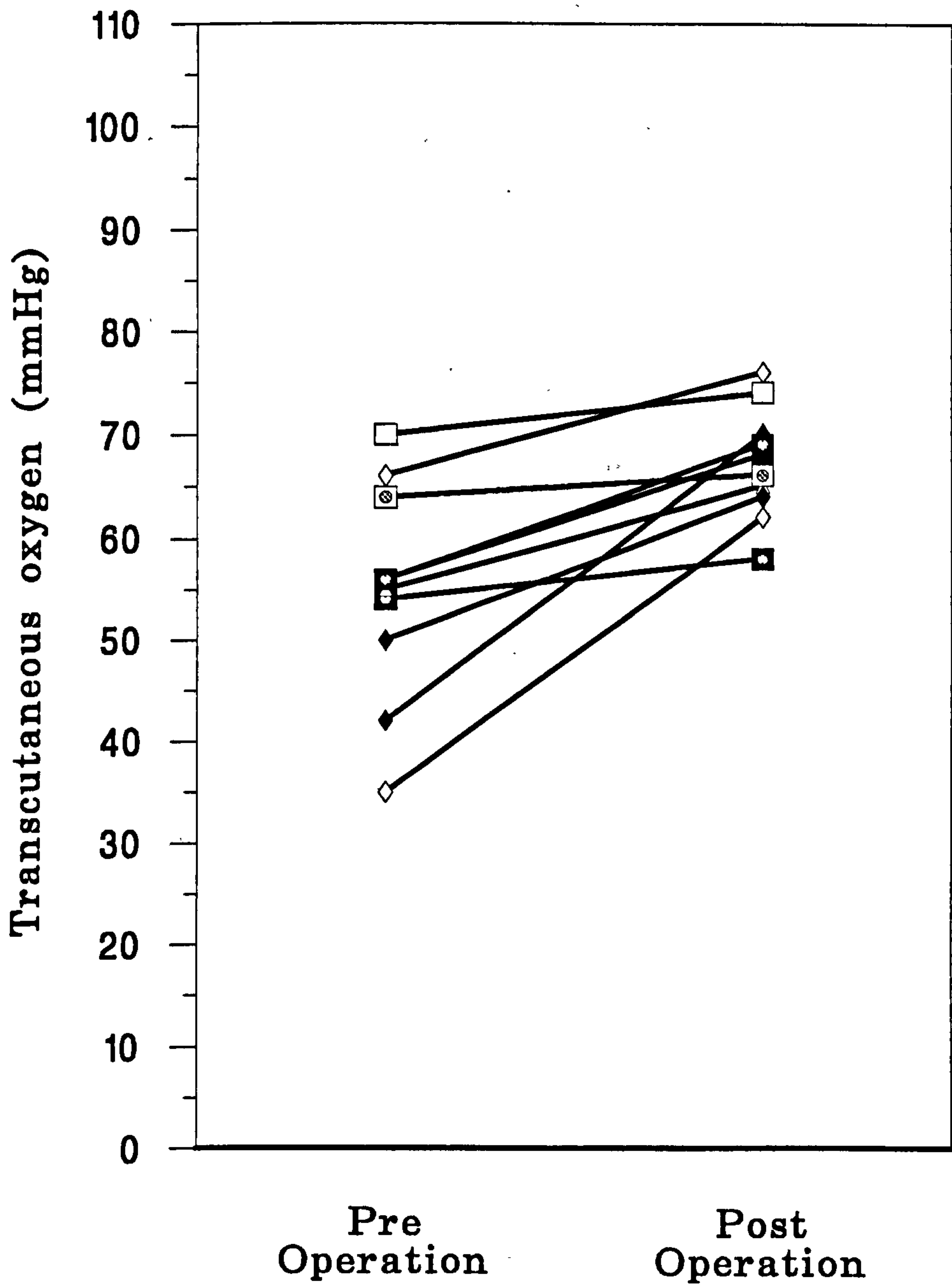


Figure 4.2. Change in peroneal conduction velocity following revascularisation in non-diabetic patients.

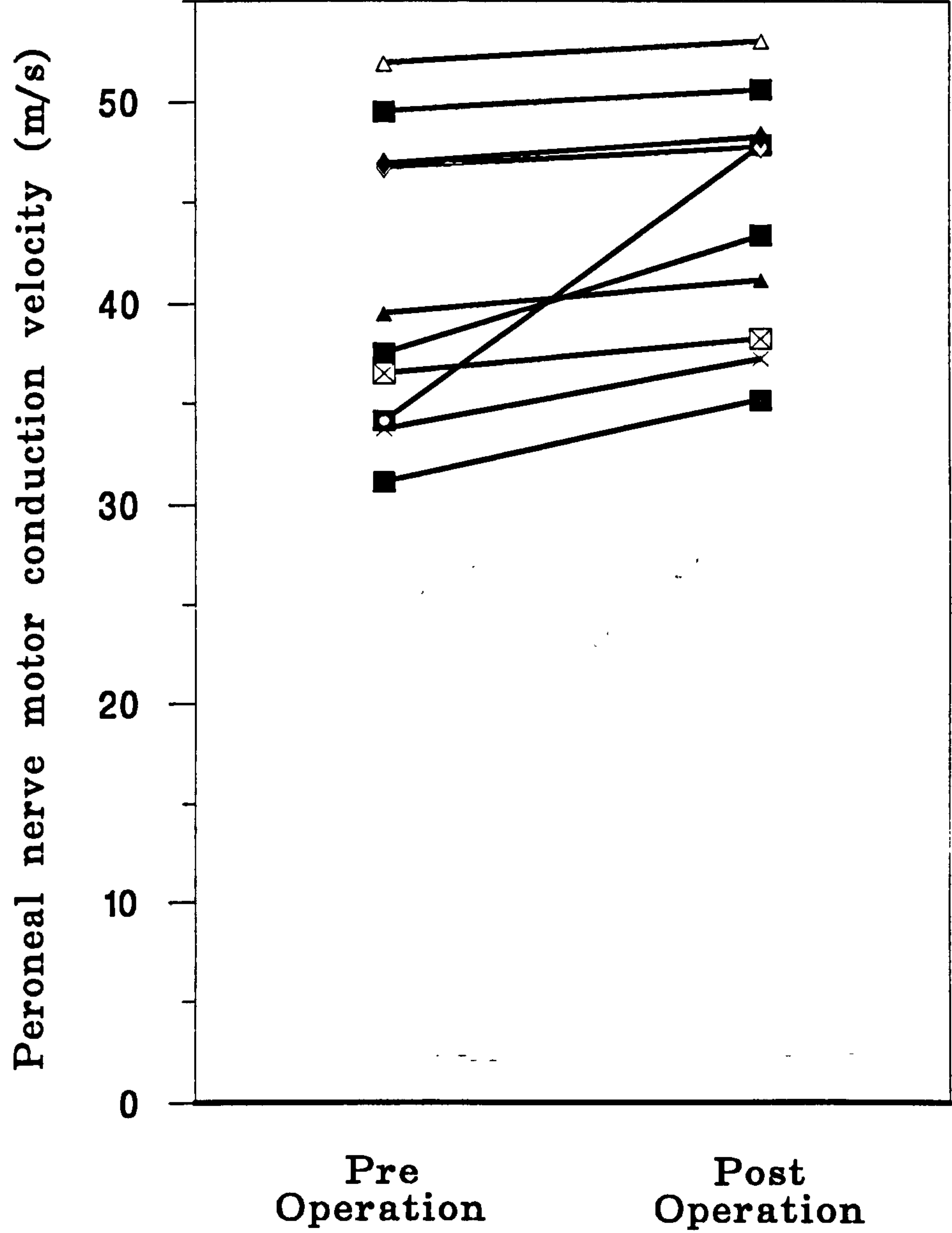


Figure 4.3. Graph showing improvement in tissue oxygenation of the foot in the diabetic patients following revascularisation.

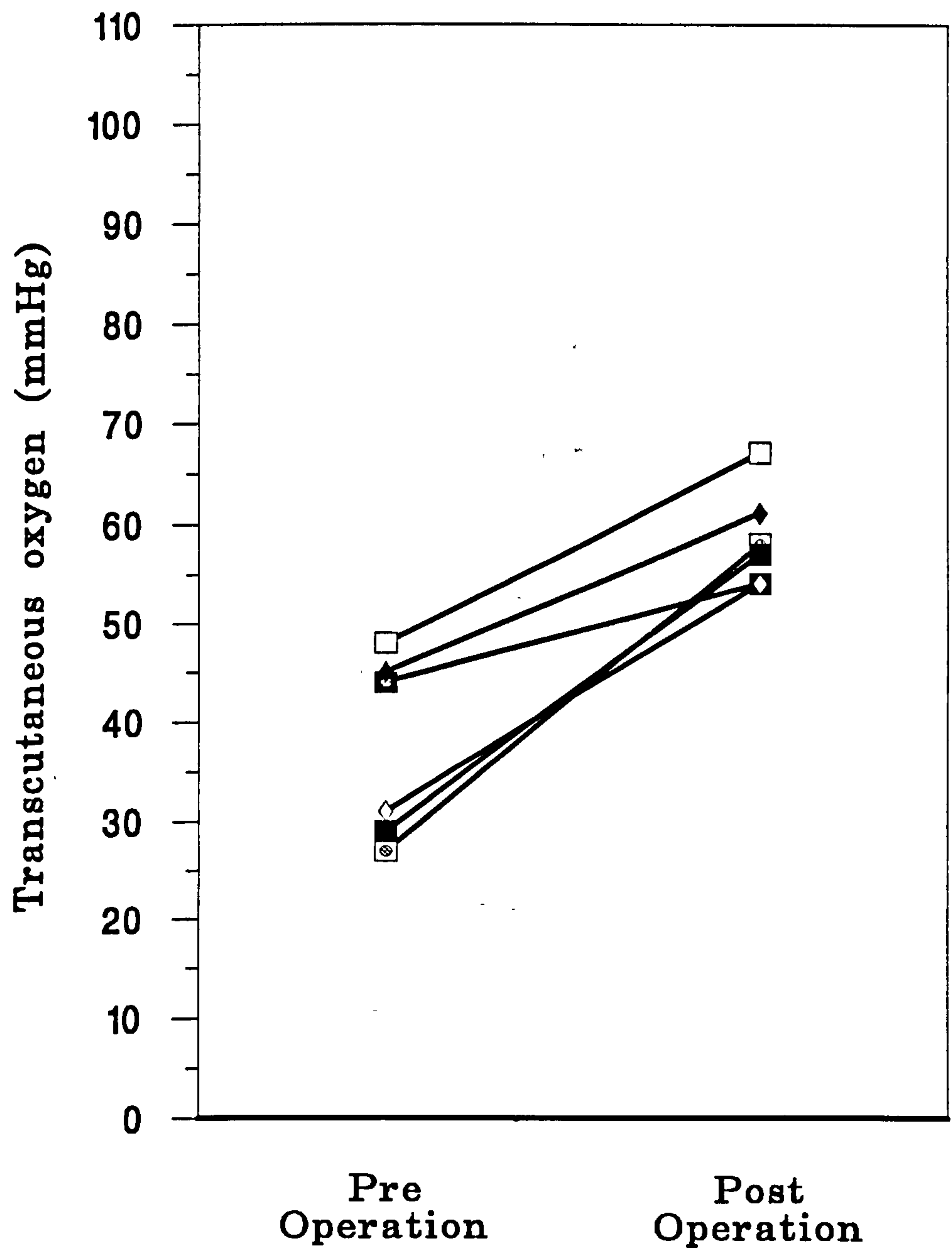
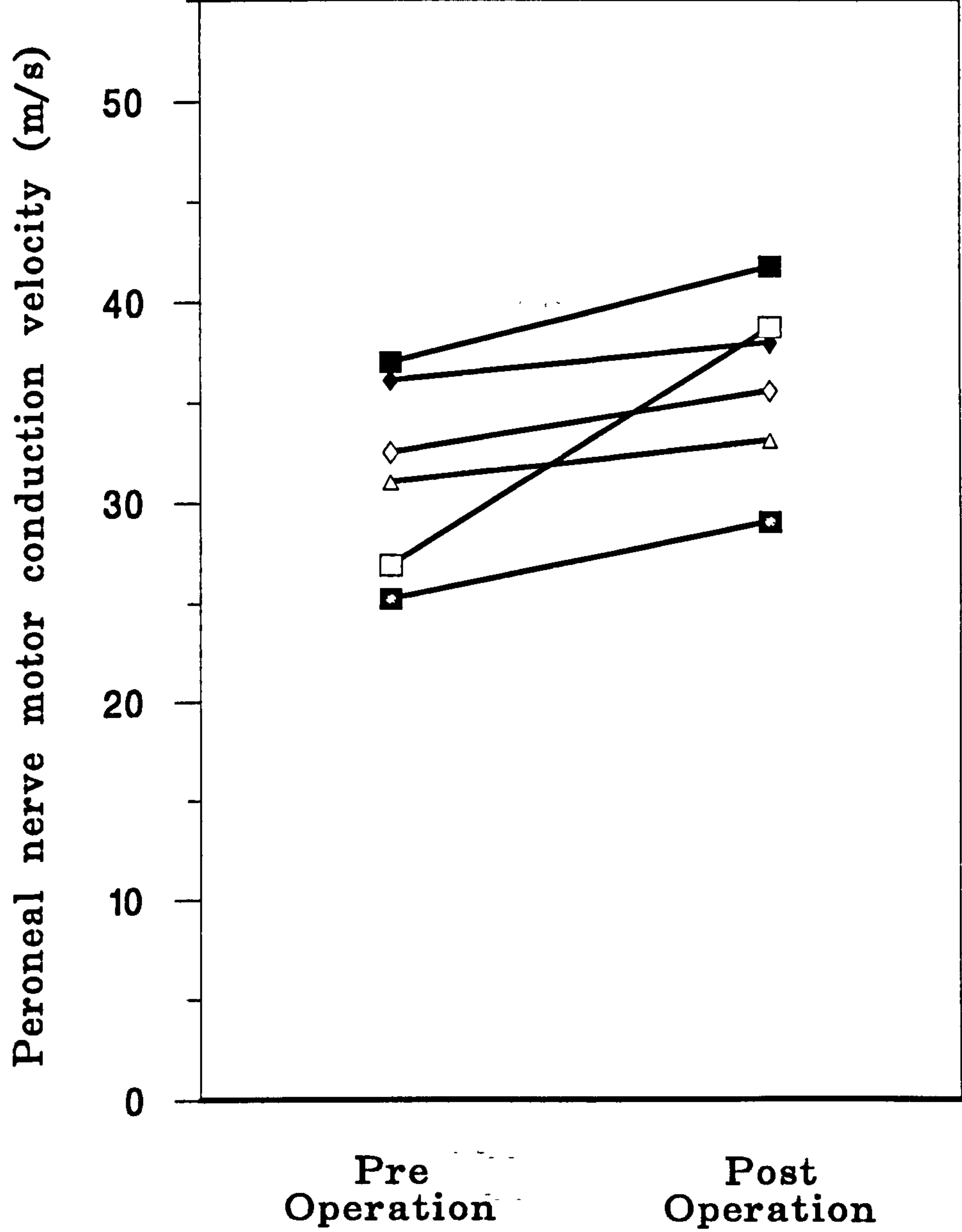


Figure 4.4. Changes in peroneal conduction velocity in the diabetic patients following revascularisation



Chapter Five

Discussion of Results in Section One

Discussion

The evidence that microvascular flow and endoneurial hypoxia are implicated in the development of peripheral neuropathy is increasing in both animal and human studies (Introduction, pages 17-23). The clear relationship between tissue oxygenation and nerve function observed in this study supports the view that hypoxia is implicated in the aetiopathogenesis of peripheral neuropathy and extends the findings of Ram et al. (1991). In their study, clinical correlates of peripheral vascular disease were found to correlate with peroneal conduction velocity, and again differences were found between each leg. These findings are, however, apparently in contrast with other studies which have shown that blood flow in the neuropathic diabetic foot is actually increased (Edmonds et al. 1982). One possible explanation for this is the considerable evidence of arterio-venous shunting (Ward et al. 1983), including a high oxygen concentration in dorsal foot veins (Boulton et al. 1982) in the diabetic foot. Arterio-venous shunting has also been demonstrated in the blood supply of the sural nerve of diabetic patients with neuropathy (Tsfaye et al. 1990) and it is likely that the lower skin oxygen tensions measured in this study reflect shunts away from nutritive flow (Boulton et al. 1982, Edmonds et al. 1982, Ward et al. 1983, Rayman et al. 1986, Tsfaye et al. 1990). Other explanations for a lower transcutaneous oxygen tension in neuropathic patients might include basement membrane thickening, which has been reported in the skin of neuropathic patients as part of a wider microvasculopathy, and would act as a barrier to diffusion. The role of adverse rheological changes in reducing transcutaneous oxygen measurements in diabetic patients is discussed below.

The absence of a direct correlation between changes in temperature and nerve function has been demonstrated previously in diabetic patients using subcutaneous thermometers and was thought to reflect the failure of endoneurial blood flow to improve in diabetic sural nerve (Tsfaye et al. 1992). It is also possible that the autotomy described in these patients (Watkins and Edmonds 1983) removes the

association between temperature and blood flow in diabetic patients. The high basal temperature values in each group of subjects also suggests that the warm room in which the studies were performed reduced any differences in temperature between the neuropathic and non-neuropathic subjects, thus removing any possible correlation that might have existed in a cooler room.

This study reports abnormalities in a number of rheological and microcirculatory parameters in diabetic patients with peripheral neuropathy when compared to matched non-diabetic control subjects. The lack of a statistically significant difference in all but red cell aggregation and fibrinogen concentration, between diabetic patients with and without peripheral neuropathy, is in keeping with the other comparable studies which have examined rheological parameters in diabetic peripheral neuropathy (MacRury et al. 1991, MacRury et al. 1993a). However, in these studies too, the neuropathic diabetic patients contributed the major component of the differences reported between 'all diabetic patients', which comprised the neuropathic and non-neuropathic patients grouped as a whole, and the non-diabetic control subjects. In addition, in these studies the control groups were also younger than the diabetic groups. The degree of neuropathy in the diabetic patients in the studies of MacRury et al. was also milder, with a mean peroneal conduction velocity of 40.7 ms^{-1} (MacRury et al. 1991). In the study of Ford et al. (1992) the diabetic patients had the same degree of neuropathy as the patients in this study, but the methods used were not entirely comparable and no non-diabetic control group was included for comparison.

Studies of haemorheology in patients with the microvascular complications of diabetes have generally reported mixed results (MacRury and Lowe 1990). The evidence for increased whole blood viscosity in diabetes is generally accepted, however most studies have examined whole blood viscosity in patients with established complications such as nephropathy (Gordge et al. 1990) (although the

changes were similar in diabetic and non-diabetic patients with renal failure) or retinopathy (Lowe et al. 1980a, 1986). In addition, no significant increase in whole blood viscosity has been found in patients with background retinopathy when compared to diabetic patients with no evidence of retinopathy (MacRury and Lowe 1990) or in patients with and without microalbuminuria (Jay et al. 1991). Similarly, MacRury et al. (MacRury et al. 1991) reported that there was no significant difference in whole blood viscosity at high or low shear rates in diabetic patients with and without neuropathy, either at native haematocrit or at a standardised haematocrit, although non-statistically significant increases were reported at both the shear rates examined in this study (98 s^{-1} , 0.98 s^{-1}). This study also included patients with other microvascular complications in the neuropathic and non-neuropathic control groups, and smokers in all groups, which might have influenced the findings.

Most recent, widely published studies, confirm that plasma viscosity is increased in diabetic patients (MacRury and Lowe 1990), a finding which was first reported by Cogan et al. in patients with retinopathy (Cogan et al. 1961). This increase is generally in association with increased fibrinogen levels in diabetic patients (Ganda and Arkin 1992). However, other recent studies by MacRury et al. have shown an increase in plasma viscosity without an increase in fibrinogen (MacRury et al. 1991) and no difference in plasma viscosity between diabetic patients and controls (MacRury et al. 1993a).

MacRury et al. (1993b) have also examined red cell aggregation in diabetic patients in relation to cardiovascular disease. In mixed groups of type 1 and type 2 diabetic patients with and without microvascular complications red cell aggregation was increased compared to control subjects. There are however, few studies of red cell aggregation in the absence of microvascular disease or poor control (MacRury and Lowe 1990). The patients in this study had average diabetes control and were well matched to the non-diabetic control subjects. The absence of a significant difference in

rheological parameters between non-diabetic control subjects and uncomplicated diabetic patients may therefore be a new finding.

The reduced transcutaneous oxygen tensions and laser Doppler flux in the neuropathic diabetic patients, measured at 44 °C is in keeping with previous studies (Eickhoff and Jacobsen 1980, Railton et al. 1983). In particular, this may reflect microvascular sclerosis with a resultant reduction in the maximal hyperaemic response to heating in neuropathic patients, and/or an increase in arterio-venous shunting (Flynn and Tooke 1992). A recent review of rheological changes in diabetes suggested that the increase in blood viscosity, particularly at lower shear rates, reported in diabetes might be reflected in reduced flow in the post-capillary venules (MacRury and Lowe 1990). It was also suggested that this might in turn promote capillary stasis, leading to local hypoxaemia, and contribute to microvascular complications. This hypothesis is supported by the correlations between nerve conduction and tissue oxygenation and rheological parameters found in this study. When these correlations are taken together with the association between fibrinogen concentration and basement membrane thickening reported in the study of Ford et al. (1992), rheological mechanisms might also provide a further explanation for the reduced endoneurial and transcutaneous oxygen levels reported in patients with neuropathy (Railton et al. 1983). The finding of reduced prostacyclin and reduced prostacyclin / thromboxane ratio in neuropathic diabetic patients is in keeping with previous reports (Moncada and Vane 1979) and might also contribute to the reduced capillary flow in neuropathic patients suggested by the results of this study.

As well as adverse rheological changes, a reduced fibrinolytic response has been implicated in the increased prevalence of macrovascular disease in diabetic patients (Meade et al. 1980). The attenuated fibrinolytic response in diabetic patients has been related to hyperinsulinism (Juhan-Vague et al. 1989), and therefore may reflect insulin treatment in the type 1 patients and insulin resistance in the type 2

patients, although obesity and hypertension are believed to be the primary abnormalities leading to impaired fibrinolytic function (Juhan-Vague et al. 1987, Auwerx et al. 1988, Potter van Loon et al. 1990, Grant 1991, Landin et al. 1991). The current findings would support the hypothesis that basal fibrinolysis is reduced in diabetic patients (Small et al. 1987), and would agree with the assumption that this is due to increased body mass and higher blood pressure levels in diabetic patients.

Stimulated fibrinolysis was also reduced in the diabetic patients compared to controls. There was a trend to a lower stimulated fibrinolytic response in non-neuropathic patients with a significantly impaired response in the neuropathic diabetic patients. This result implies that neuropathic patients may have a reduced ability to clear microthrombi in the microcirculation. Such microthrombi would be more likely to form in neuropathic patients due to the greater level of adverse rheological changes and higher concentrations of fibrinogen. Impaired clearance of microthrombi has been suggested by the findings of thrombi within endoneurial capillaries, and the suggestion that they might then play a part in the microvascular abnormalities, including capillary closure, that have been reported in diabetic neuropathy (Simpson 1988). Indeed the study of Ford et al. (1992), which demonstrated that capillary luminal area was inversely proportional to fibrinolytic activity adds significant support to such a concept.

Further evidence of microvascular abnormalities in diabetic patients has been supplied by studies of microvascular flow using laser Doppler techniques. Rayman et al. (1986) examined the response of skin to thermal and mechanical trauma and found that reactive hyperaemia was reduced in diabetic patients with microvascular complications in the foot and the abdominal wall, where neuropathy is unlikely to have directly influenced the response. Similar work from the Exeter group (Shore et al. 1991, Flynn and Tooke 1992) and the King's group (Stevens M et al. 1992a,b, Watkins 1992) has confirmed this finding, and work from Exeter has shown that this

reduced hyperaemic response can be found in children with diabetes prior to the development of complications (Shore et al. 1991). The finding of a reduced hyperaemic response in neuropathic patients in this thesis is therefore in keeping with previous reports. The mechanism behind this impaired response might possibly be a function of abnormalities of local neurogenic responses (Stevens M et al. 1992a), as a result of the lower prostacyclin production in diabetic patients (Moncada and Vane 1979), or, as is more likely in view of the early onset of such changes and presence of changes on the trunk, a result of microvascular sclerosis. Microvascular sclerosis may have a basis in the basement membrane thickening described in diabetes (Faris et al. 1982), and therefore, indirectly, the finding of an impaired hyperaemic response also adds to the evidence of increased microvascular dysfunction in neuropathic diabetic patients.

Revascularisation

It could be reasoned that if diabetic peripheral neuropathy is due, at least in part, to impaired endoneurial blood flow, then a reduction in blood flow or tissue oxygenation alone should result in peripheral nerve dysfunction in non-diabetic subjects. This is supported by the reports of peripheral neuropathy in hypoxic chronic obstructive airways disease patients (Nowak et al. 1990), morphological changes in the sural nerve of patients with peripheral vascular disease (Rodriguez-Sanchez et al. 1991) and the observations of the effects of proximal shunts described on nerve function (Sladky et al. 1991, Wilbourn et al. 1983, Knezevic and Mastalgia 1984, Riggs et al. 1989). The studies which examined patients with unilateral femoral artery thromboses undergoing reconstructive surgery were used as a model of impaired endoneurial flow alone in the development of peripheral nerve dysfunction, and to study effects of its improvement. Unilateral surgery allowed the contralateral (control) leg to act as a control for other variables such as smoking, changes in diabetic control or drugs. The marked increase in tissue oxygenation following surgery was

accompanied by a significant rise in peroneal conduction velocity in both diabetic and non-diabetic patients. This is in contrast to a previously published study by Hunter et al. (1988). However in the previous study, there were mixed groups of diabetic and non-diabetic patients and the degree of vascular impairment was greater, with half of the femoro-popliteal reconstructions being performed for limb salvage, at which stage irreversible changes are likely to have occurred. Also this study looked at patients after a year, by which time restenosis may have occurred in a number of patients.

The absence of a significant rise in skin temperature following reconstructive arterial surgery may reflect the half hour or more of equilibration in a warm room that was allowed before the measurements were taken.

Although not significant in these relatively small numbers of patients, there was a fall in transcutaneous oxygen tension, peroneal nerve MCV and skin temperature in the control leg of the non-diabetic patients following surgery. This may reflect a form of steal phenomenon, as peripheral vascular disease is usually bilateral, although asymmetrical, and the side of the bypass operation is directed by clinical indications.

Conclusions

In summary, well matched groups of otherwise uncomplicated, non-smoking, neuropathic diabetic patients have been demonstrated to have adverse changes in haemorheological, fibrinolytic, and microvascular parameters, which are significant when compared to non-diabetic control subjects. These changes add further evidence to support the role of the microvascular dysfunction in the pathogenesis of diabetic neuropathy, in keeping with the other principal long term complications of diabetes (Simpson 1988, Valensi et al. 1991), and suggest that rheological abnormalities are found in diabetic neuropathy in the absence of other complications.

In addition, these studies demonstrate that there is a significant relationship between tissue oxygenation and peripheral nerve function in diabetic and non-diabetic patients, and that in both, improving tissue oxygenation is accompanied by a significant improvement in nerve function. These findings lend support to the hypothesis that endoneurial hypoxia is implicated in the aetiopathogenesis of diabetic peripheral neuropathy, and suggest that therapeutic strategies to improve blood flow, particularly in early neuropathy, should be considered in diabetic patients.

Section Three

The Effects of Peripheral Neuropathy on the Diabetic Foot

Chapter Six

A Radiographic Survey of Abnormalities in the Feet of Neuropathic and Non-Neuropathic Diabetic Patients and Matched Non-Diabetic Controls

Patients and Methods

A total of 137 diabetic patients and 50 age and sex matched healthy control subjects (C) were included in this study. Their characteristics are detailed in Table 6.1. In summary, there were 54 diabetic patients with neuropathy and a history of foot ulceration (U), 40 with neuropathy without foot ulceration (N) and 43 with no clinical neuropathy (NN). No patient had a foot ulcer at the time of study.

Diabetic subjects were chosen at random from the appropriate clinical groupings as identified on the computer database at the Manchester Diabetes Centre. In total, 179 patients were known to have had neuropathic ulceration. In 1988-1989, 520 consecutive patients had their vibration perception measured, and of these, 177 had a vibration perception threshold greater than 30V and no history of ulceration, and 260 had a vibration perception threshold of less than 25V. These patients formed the patient pool for recruitment. The diabetes centre is a hospital based diabetes clinic and, as with most studies of this type may not be representative of the distribution of diabetes within the general population. Therefore, this study examined comparisons between groups and relationships within them rather than stating absolute prevalence rates for abnormalities within the diabetic population as a whole.

Normal non-diabetic control subjects were taken from the age-sex register of a general practice within the referral area of the diabetes centre. These patients had no history of renal failure, alcoholism or other cause of neuropathy and no symptoms of intermittent claudication.

Vibration perception was measured using a Biothesiometer (Biomedical, Newbury, Ohio, USA) and was recorded as the mean value of five sites in each foot. Patients were designated as neuropathic if they had a mean vibration perception threshold of greater than 30V. A previous history of foot and leg ulcers and known fractures was sought by direct questioning.

HbA1c (laboratory normal <8%) and serum creatinine were determined on all diabetic patients.

The physical characteristics of the patients and control subjects are shown in Table 6.1. After recruitment to the study the diabetic neuropathic patients with previous foot ulceration were found to have a longer duration of diabetes and an excess of type 1 (insulin-dependent) diabetic patients than the non-neuropathic diabetic patients ($p<0.05$). Type 1 diabetic patients had an overall significantly longer duration of diabetes than the type 2 diabetic patients, median 26 (20-35 interquartile range) years vs. 11 (8-19) years ($p<0.01$). The non-ulcer neuropathic diabetic patients were older than the other diabetic groups and the non-diabetic control subjects ($p<0.05$), (Table 6.1). The median VPT of the neuropathic patients with a history of foot ulceration was similar to that of the neuropathic non-ulcer patients, despite the latter patients being significantly older.

The diabetic patients as a whole, and the non-neuropathic patients (NN) in particular, were well matched for age and gender ratio to the non-diabetic (C) controls, (Table 6.1).

The brachial systolic blood pressure was measured in the right arm and the ankle systolic pressures were measured at the dorsalis pedis artery on each foot using an handheld Doppler ultrasound (Oxford Sonicaid Ltd., Oxford, UK). The mean ankle systolic and ankle-brachial pressure index was used in the analyses. Foot pulses were assessed by palpation: their absence was recorded if no pulses were palpable in either foot.

Table 6.1. Physical characteristics of the subject groups

	n	M:F	Median age (years)	Inter quartile range	VPT (Volts)	Inter quartile range	Diabetes Type I/II	Diabetes duration (years)	Inter quartile range	HbA1 (%) ^a	Inter quartile range	Creatinine (μmol/l)	Inter quartile range
Ulcer neuropathic (U)	54	41:13 ^b	60.5	50.5-67.0	48.0 ^c	40.0-51.0	24:30 ^b	19.5 ^e	9.9-29.2	10.6	9.1-13.1	113.5 ^e	95.0-192.0
Neuropathic no ulcer (N)	40	27:13	68.0 ^b	62.2-73.0	45.8 ^c	36.7-51.0	16:24	14.0 ^f	8.0-28.0	10.6	9.3-11.8	96.0 ^g	89.5-110.5
Non-neuropathic (NN)	43	29:14	60.5	52.0-68.5	17.6 ^d	12.3-21.1	14:29	14.0 ^f	5.0-20.0	10.0	8.4-12.0	90.0 ^g	79.9-99.0
All DM	137	97:40	63.0	54.0-69.0	40.0	21.8-49.9	51:86	15.5	8.0-26.0	10.5	9.1-12.4	97.0	87.0-120.5
Non diabetic controls (C)	50	35:15	62.5	53.7-70.0	15.2 ^d	11.0-24.2							
All	187	132:55	63.0	54.0-69.0	34.6	16.4-47.6							

^a No significant difference between all groups.

^b p<0.05 N vs all other groups.

^c p=NS U vs N, p<0.01 U or N vs NN or C.

^d p=NS NN vs C.

^e p<0.05 U vs N or NN.

^f p=NS N vs NN.

^g p<0.05 N vs NN.

Radiographic technique and analysis

Weight-bearing (standing) true dorsi-plantar and lateral radiographs were taken of each foot using the standardised protocol described in the methods, Chapter 2, page 69. All the radiographs were read by a single radiologist (Dr Judith Adams) without knowledge of the subjects' clinical category. The presence and grade of bone and joint changes were assessed as detailed in the methods section, pages 70-77. Soft tissue calcification was classified as described below.

Medial arterial calcification was defined as parallel tramline calcification and, if present, was graded as mild or severe according to previously published criteria (Morrison and Bogan 1929). Absent calcification was scored as 0, mild calcification as 1 and severe as 2. Medial arterial calcification was assessed in four soft tissue regions on dorsum and plantar aspect of each lateral radiograph, these were the ankle, hind foot, mid-foot and metatarsals. As the interdigital and intermetatarsal arteries are not normally visible on lateral views, three regions on the A-P projections, the toes, metatarsals and mid-foot were also assessed.

For the purposes of analysis the calcification data were analysed in two ways. The total amount of medial arterial calcification (total arterial calcification score) was assessed as the sum of the scores in each region, giving a total maximum score of 44 if severe calcification was present in all eleven regions of each foot. Amputees were scored as twice the score of the remaining intact foot. For comparing the regions and modelling the prevalence of calcification, the presence or absence of calcification on any view in each region of either foot was used.

Statistical analysis

General

All the standard statistics, other than Fisher's exact test, were performed using Minitab software (Minitab Inc, State College, Pa., USA). The majority of the data was not normally distributed and Mann-Whitney U tests have been used throughout for the standard comparisons between groups. Chi-squared tests have been used to assess the distribution of gender and diabetes type between groups and for tests of associations except when the number of occurrences of the abnormality was small (expected value < 5), when Fisher's Exact test using SAS software (SAS Institute Inc., Cary, N.C., USA) was used. Mantel-Haentzel risk ratios were also calculated where appropriate. Spearman Rank correlations were used to assess the inter-relationships between parameters.

Medial arterial calcification data

The ceiling effect of a maximum VPT of 50 V and a skewed distribution of calcification would artificially inflate the correlations in the diabetic groups. All correlations therefore include only those subjects with a measurable VPT and/or a minimum calcification score of 1, this left 86/137 of the diabetic patients in correlation analyses.

Within the control group the total calcification score, median 0.0 (range 0-10, inter quartile range 0.0-0.0) was markedly skewed to the left, with 40 of the 44 subjects scoring 0. Therefore correlations of the total arterial calcification score and other parameters using this group were not performed.

An ordered categorical model was fit to the data (Agresti 1990) in order to determine the existence and nature of any ordered progression in the prevalence of calcification along the axis of the foot (ankle to toe) and between groups of patients. The presence or absence of medial arterial calcification in a given region represented the dichotomous variable of interest. The ordered categorical data analysis was performed using 'Proc Catmod' in SAS software (SAS Institute Inc., Cary, N.C., USA). The complete absence of calcification in the toe region of the non-diabetic control subjects resulted in a zero cell for that data point. As this caused a singularity, which would not allow the program to run, the value 0.00001 was placed in this cell. Running the model with values as high as 0.01 did not alter the result and therefore 0.00001 was felt to be as near zero as to be insignificant in its effects on the model.

A statistical model was fit to this ordered data to reveal any trends which may have been suppressed by the underlying variability in the data. The log odds of the probability of calcification was modelled using nominal group effects (U, N, NN, C)

and ordinal region effects (heel, hind-foot, mid-foot, metatarsals, toes), with an indicator term for calcification in the toe region.

Finally, to examine the multivariate predictors of mild and severe calcification in diabetic patients, two stepwise logistical regression models were used to predict the probability of calcification in any region of the foot. In the first model patients were allocated in two groups as those with any calcification versus those with no calcification. In the second model those with severe calcification in at least one region were contrasted with all the others. The potential predictor variables offered to the models were ankle systolic pressure, ankle pressure index, VPT, age, sex, duration and type of diabetes, HbA1 and serum creatinine. The analysis was performed using the SAS procedure 'Proc logit'. The p values for entry and exit of the model were set at 0.1. The procedure was run with and without patients with off scale VPT (>50 V) and the results were similar. The results shown below are those without these patients to remove the ceiling effect created by these patients.

Results

Bone abnormalities

There were no significant differences in any of the bone changes recorded in non-neuropathic diabetic patients (NN) and the age and sex-matched non-diabetic (C) controls (Table 6.2).

Traumatic fractures were found in 12 (22.2%) of the neuropathic patients with previous foot ulceration (U), however, 9 of the 12 patients could not recall any injury to the foot. In contrast, the three other diabetic patients, one in the non-neuropathic group and two (5%) in the neuropathic patients without foot ulceration (N) found to have traumatic fractures were all able to recall the traumatic event which caused the fracture, and seeking treatment for it. The total number of fractures in the neuropathic patients with a previous history of foot ulceration was significantly greater than in the neuropathic patients without previous foot ulceration, ($p < 0.04$ U vs. N).

The neuropathic patients without a history of foot ulceration (N) had a significantly higher prevalence of periosteal reaction than the non-neuropathic diabetic group (32.5% vs. 13.9%, $p < 0.04$). In addition, in the neuropathic foot ulcer (U) patients, the presence of bone destruction (25.9% vs. 0%, $p < 0.001$) was significantly greater than in the neuropathic diabetic patients without a history of foot ulceration.

Juxta-articular exostoses were present in approximately three quarters of all individuals examined irrespective of group. Atrophic changes (such as 'pencilling' or 'waisting') were not significantly different between all diabetic patients and controls, but pencilling was more common in the ulcer compared to non-ulcer neuropathic diabetic patients ($p < 0.03$).

Fourteen of the neuropathic diabetic patients with previous foot ulceration had an amputation of part (9 patients, 1 forefoot, 2 rays, 1 all toes, 2 hallux, 2 minor

toes, 1 metatarsal head resection) or all (5 patients, 4 below knee, 1 above) of one foot. There were no amputations in any of the other groups, ($p < 0.001$).

Table 6.2: Frequency, between group comparisons, and risk ratios (RR) of bony abnormality.

	Non-Diabetic Control subjects (C)		Non-Neuropathic Diabetic (N)		Neuropathic ulcer (NN)		Neuropathic Ulcer (U)		C vs NN		NN vs N		N vs U	
	n	%	n	%	n	%	n	%	p	RR	p	RR	p	RR
Traumatic Fractures	1	2	1	2.3	2	5	12	22.2	0.79	1.17	0.35	3.40	0.03*	5.43
Periosteal reaction	8	16	6	13.9	13	32.5	24	44.4	0.78	0.85	0.04*	2.97	0.24	1.66
Osteopaenia	11	22	10	23.3	10	25	19	35.2	0.88	1.07	0.85	1.1	0.29	1.63
Exostoses	37	74	26	60.5	32	80.0	38	70.4	0.16	0.54	0.053	2.61	0.29	0.59
Infection	0	0	0	0	0	0	2	3.7	-	-	-	-	0.51	-
Destruction	2	4	0	0	0	0	14	25.9	0.49	-	-	-	0.00*	-
Erosion	1	2	1	2.3	2	5.0	8	14.8	1.0	1.17	0.61	2.21	0.18	3.30
Waisting	8	16	9	20.9	3	7.5	10	18.5	0.54	1.39	0.08	0.31	0.13	2.8
Pencilng	1	2.0	0	0	0	0	6	11.1	1.0	-	-	-	0.03*	-
Amputation	0	0	0	0	0	0	14	25.9	-	-	-	-	0.00*	-

*significant p<0.05

Joint Abnormalities

There were no significant differences in the frequency of joint abnormalities between the non-diabetic control subjects and non-neuropathic diabetic patients.

Charcot changes were noted in 9 (16.7%) of the neuropathic ulceration group but in none of the other groups, ($p=0.009$).

Joint fragmentation and dislocation were also significantly greater in the diabetic neuropathic ulcer group ($p=0.009$ and $p=0.02$ respectively). Although failing to reach statistical significance against the non-ulcer neuropathic group, joint destruction was twice as common in the ulcer patients and three times that of the non-neuropathic patients ($p<0.05$, risk ratio 4.67 (1.22-20.3 95% confidence limits))

Abnormal joint alignment, osteophyte formation, subchondral sclerosis, and narrowed joint spaces (principally at the distal interphalangeal joints) were present in at least three-quarters of all subjects. The high prevalences observed were not significantly different between groups.

Table 6.3: Frequency, between group comparisons, and risk ratios (RR) of joint abnormality.

	Non-Diabetic Control subjects (C)		Non-Neuropathic Diabetic (N)		Neuropathic no ulcer (NN)		Neuropathic Ulcer (U)		C vs NN		NN vs N		N vs U	
	n	%	n	%	n	%	n	%	p	RR	p	RR	p	RR
Charcot change	0	0	0	0	0	0	9	16.7	-	-	0.01*	-	0.01*	-
Subchondral Sclerosis	44	88	32	74.4	35	87.5	50	92.6	0.09	0.39	0.13	2.41	0.49	1.79
Osteophytes	38	76	27	62.8	23	57.5	39	72.2	0.17	0.53	0.62	0.80	0.14	1.92
Fragmentation	1	2	2	4.6	0	0	9	16.7	0.59	2.39	0.49	-	0.01*	-
Destruction	3	6	3	6.9	5	12.5	14	25.9	1.0	1.17	0.47	1.90	0.11	2.45
Dislocation	3	6	1	2.3	2	5	12	22.2	0.62	0.37	0.61	2.21	0.02*	5.43

*significant p<0.05

Medial Arterial Calcification

Overall prevalence of medial arterial calcification

The prevalence of calcification in each region of the foot in each group of subjects is detailed in Table 6.4. There was a gradient in the distribution of medial arterial calcification with a greater prevalence at the ankle and hind foot than at the toes in all groups (Table 6.4). This is further explored in the statistical model below.

The total calcification score in the neuropathic diabetic group with a history of foot ulceration was significantly higher than the neuropathic group without foot ulceration, (U vs. N $p < 0.01$) (Table 6.5). The neuropathic diabetic patients without foot ulceration had significantly higher total calcification scores than the non-neuropathic diabetic control subjects, (N vs. NN $p < 0.01$). There was no significant difference between the total calcification score in the non-neuropathic diabetic and non-diabetic groups, ($p = 0.25$).

Table 6.4. Prevalence of medial arterial calcification in the foot by subject group and region (%)

	n	Ankle	Hind Foot	Mid Foot	Metatarsals	Toes
Ulcer neuropathic (U)	54	78.8	75.0	69.2	69.2	40.4
Neuropathic no ulcer (N)	40	61.9	47.6	50.0	40.5	23.8
Non-neuropathic (NN)	43	25.0	29.5	16.0	22.7	6.8
Non diabetic controls (C)	50	22.5	6.0	10.0	6.0	0.0

Table 6.5. Total calcification score in each subject group

	Mean total calcification score	Median total calcification score	Inter quartile range
Ulcer neuropathic (U)	18.46	18.0 ^a	3.0 - 31.0
Neuropathic no ulcer (N)	6.83	2.0 ^b	0.0 - 13.0
Non-neuropathic (NN)	4.3	0.0 ^c	0.0 - 3.0
Non diabetic controls (C)	0.7	0.0	0.0 - 0.0

^a p<0.01 U vs N.

^b p<0.01 N vs NN.

^c p=NS NN vs C.

Duration and type of diabetes. Sex and age

The total calcification score correlated with the duration of diabetes, $r = 0.32$ $p < 0.01$. No significant association was found between age and total arterial calcification score, either in the diabetic patients alone, $r = -0.18$ $p = \text{NS}$, or in the total group of all the diabetic patients and control subjects combined, $r = -0.01$ $p = \text{NS}$.

In the diabetic patients total arterial calcification score was significantly higher in type 1 (insulin-dependent) diabetic patients than in type 2 (non-insulin-dependent) diabetic patients in all groups, overall median score 13.0 (1.5-28 inter quartile range) vs. 1.0 (0-14), $p < 0.01$. The prevalence of medial arterial calcification was equal in type 1 (insulin-dependent) diabetic patients and type 2 (non-insulin-dependent) diabetic patients in each group. Type 1 (insulin-dependent) diabetic patients in each group had a longer known duration of diabetes than type 2 (non-insulin-dependent) diabetic patients, overall median duration 20 vs. 11.5 years ($p < 0.01$).

There was no significant difference in total calcification score between males and females in the diabetic patient groups, 4.0 (0-20.5) vs. 2.5 (0-18.8), $p = \text{NS}$.

Vibration perception threshold

There was no significant difference between the VPT of the ulcer and non-ulcer neuropathic diabetic patients, median 48.0 (40-51 inter quartile range) Volts vs. 45.8 (36.7-51) Volts, $p = \text{NS}$ and no difference between the non-neuropathic diabetic and non-diabetic groups, 17.6 (12.3-21.1) Volts vs. 15.2 (11.0-24.2) Volts, $p = \text{NS}$, nor there were more 'off-scale' VPT readings in the neuropathic with foot ulceration group (16 vs. 11, $p = \text{NS}$). By definition the neuropathic groups had significantly higher VPT than the non-neuropathic and control groups (Table 6.1).

In the diabetic patients VPT correlated with total calcification score, $r = 0.35$ $p < 0.01$. An off scale VPT (51) was associated with a higher prevalence of arterial calcification than a measurable VPT (< 50), (Chi squared test, $p < 0.01$). It predicted the presence of calcification with a specificity 96%, sensitivity 29.1%, and positive predictive value (ppv) 92.6%.

Serum creatinine and HbA1

Total calcification score was significantly associated with serum creatinine in the diabetic patients, $r = 0.41$ $p < 0.01$. Serum creatinine was significantly higher in the ulcer neuropathic diabetic group than the other diabetic groups. The median creatinine in the non-ulcer neuropathic group was significantly higher than the non-neuropathic group (Table 6.1). A serum creatinine of greater than $130 \mu\text{mol l}^{-1}$ was not significantly associated with an excess of MAC ($p = \text{NS}$) but a creatinine of $> 150 \mu\text{mol l}^{-1}$ was, ($p < 0.01$, sensitivity 23.5%, specificity 94%, ppv 87%).

HbA1 did not correlate with total arterial calcification score, $r = 0.05$ $p = \text{NS}$ and was not significantly different between groups (Table 6.1).

Ankle and brachial systolic pressures

The ankle and brachial pressures are detailed in Table 6.6. The brachial systolic blood pressure was significantly higher in the group of neuropathic diabetic patients with a history of foot ulceration than the non-neuropathic diabetic group and the non-diabetic group but not the diabetic patients without previous foot ulceration ($p=NS$ U vs. N, $p<0.01$ U vs. NN or C). The brachial systolic blood pressure did not correlate with calcification score in the diabetic patients, $r = 0.09$ $p=NS$.

The ankle systolic pressure was significantly higher, ($p<0.01$), in the neuropathic diabetic patients with previous foot ulceration than in any of the other groups. None of the other groups differed significantly. The median ankle brachial pressure index did not differ significantly between the four groups. In the diabetic patients ankle systolic blood pressure correlated significantly with total arterial calcification score, $r = 0.40$ $p<0.01$ as did the ankle brachial pressure index, $r = 0.35$ $p<0.01$.

The specificity and sensitivity of ankle systolic blood pressure to detect the presence of medial arterial calcification is shown in Figure 6.1. An ankle systolic pressure of 190 mmHg has a 90% specificity for the presence of MAC, but with the low sensitivity (43.2%), 56.8% of patients with MAC had ankle systolic pressures below this level. A third of all patients with MAC in this series had an ankle systolic pressure below 150 mmHg and an ankle pressure index <1.0 .

The diabetic patients with a history of foot ulceration had a higher prevalence of patients with both foot pulses absent than all other groups, but this did not reach statistical significance (Table 6.6).

Table 6.6. Systolic pressure measurements, ankle-brachial pressure indices and absent foot pulses in each group

	Median brachial systolic (mmHg)	Interquartile range	Ankle systolic (mmHg)	Interquartile range	Ankle brachial pressure index	Interquartile range	Patients with absent foot pulses
Ulcer neuropathic (U)	160 ^c	146-183	195 ^b	142-248	1.16 ^a	0.92-1.45	6 (11%) ^a
Neuropathic no ulcer (N)	155	134-170	158	120-190	1.00 ^a	0.80-1.18	2 (5%) ^a
Non-neuropathic (NN)	150	136-156	162	150-176	1.09 ^a	1.00-1.20	3 (7%) ^a
All DM	156	140-170	168	139-196	1.08	0.93-1.27	11 (8%)
Non diabetic controls (C)	150	128-170	160	141-177	1.10 ^a	0.94-1.25	1 (2%)
All	151	136-170	166	140-191	1.09	0.93-1.27	12 (6%)

^a No significant difference between all groups.

^b $p < 0.05$ U vs all other groups.

^c $p = \text{NS}$ U vs N, $p < 0.01$ U vs NN or C.

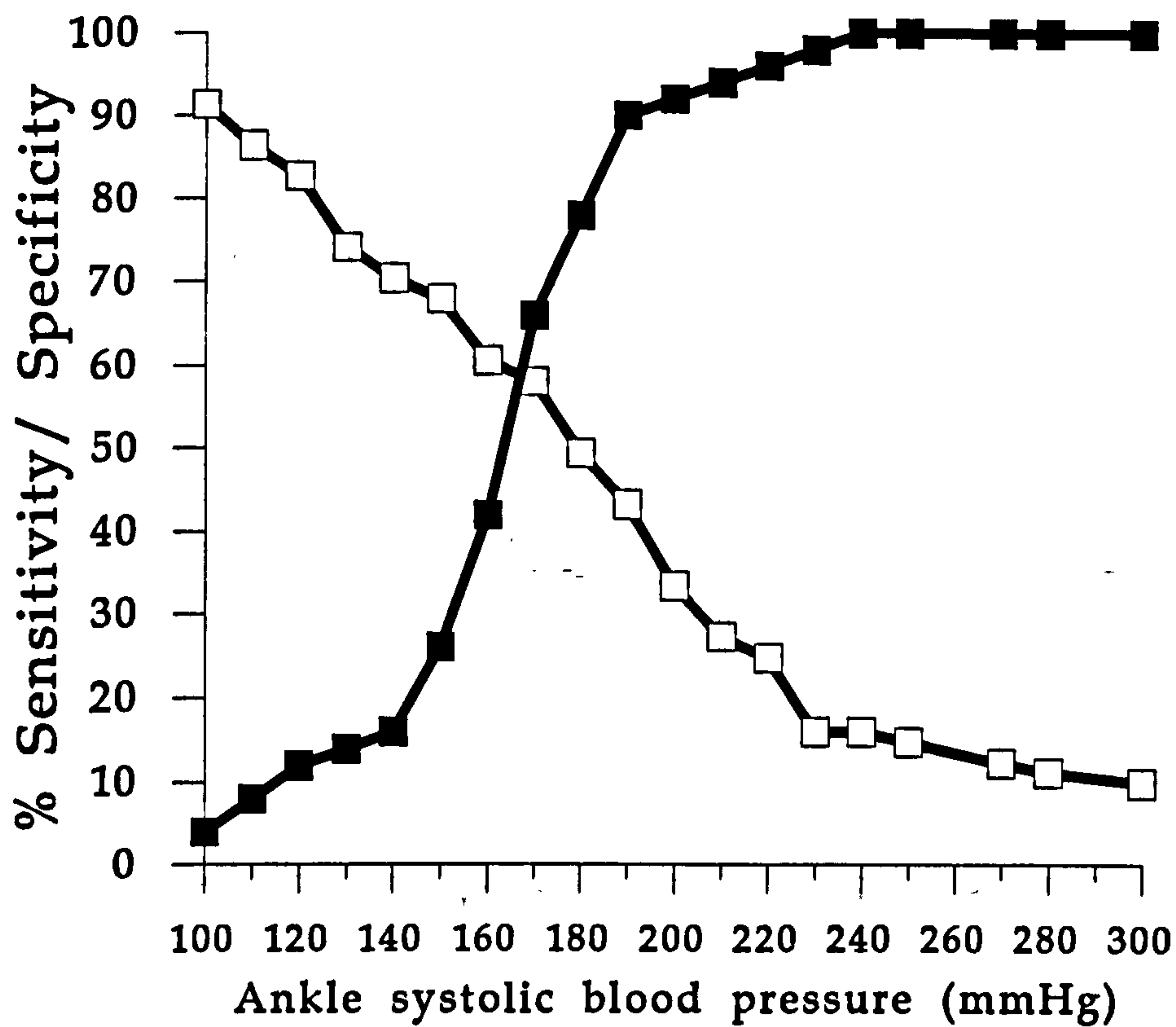


Figure 6.1. Sensitivity / Specificity of a given level of ankle systolic pressure to infer the presence of medial arterial calcification.

The graph demonstrates that arterial calcification is present in 100% of patients with ankle pressures >240 mmHg, but may also influence arterial pressures below 100 mmHg.

□ Sensitivity
 ■ Specificity

Model of the distribution of medial arterial calcification

The statistical model was fit to the entire data set and retained the structure of the data whilst suppressing the underlying variability. The Goodness of Fit statistic $p=0.273$ demonstrated no significant difference between the model and the data, i.e. a good fit (usual criterion $p>0.10$). The predicted probabilities of calcification for each region of the foot in each group are shown in Figure 6.2.

A significant difference in odds of calcification was found between neuropathic diabetic patients with and without previous foot ulceration ($p<0.01$). There was no significant difference between the predicted probability of calcification in non-neuropathic patients than in non-diabetic control subjects ($p=0.09$). Neuropathic diabetic patients had a significantly higher probability of calcification than non-neuropathic (diabetic or control) patients ($p<0.001$).

There was a significant association between odds of calcification and region of foot. ($p<0.001$). The odds of calcification was found to increase by a multiple of 1.16 (1.07-1.26 95% confidence intervals) for each region nearer the ankle. In addition to this ordered effect of region, a significant toe effect was noted ($p<0.0001$) which decreased the odds of calcification by an additional factor of 0.42. Overall, the predicted odds of calcification in the ankle were 4.35 (2.94-6.43) times greater than in the toes ($p<0.001$).

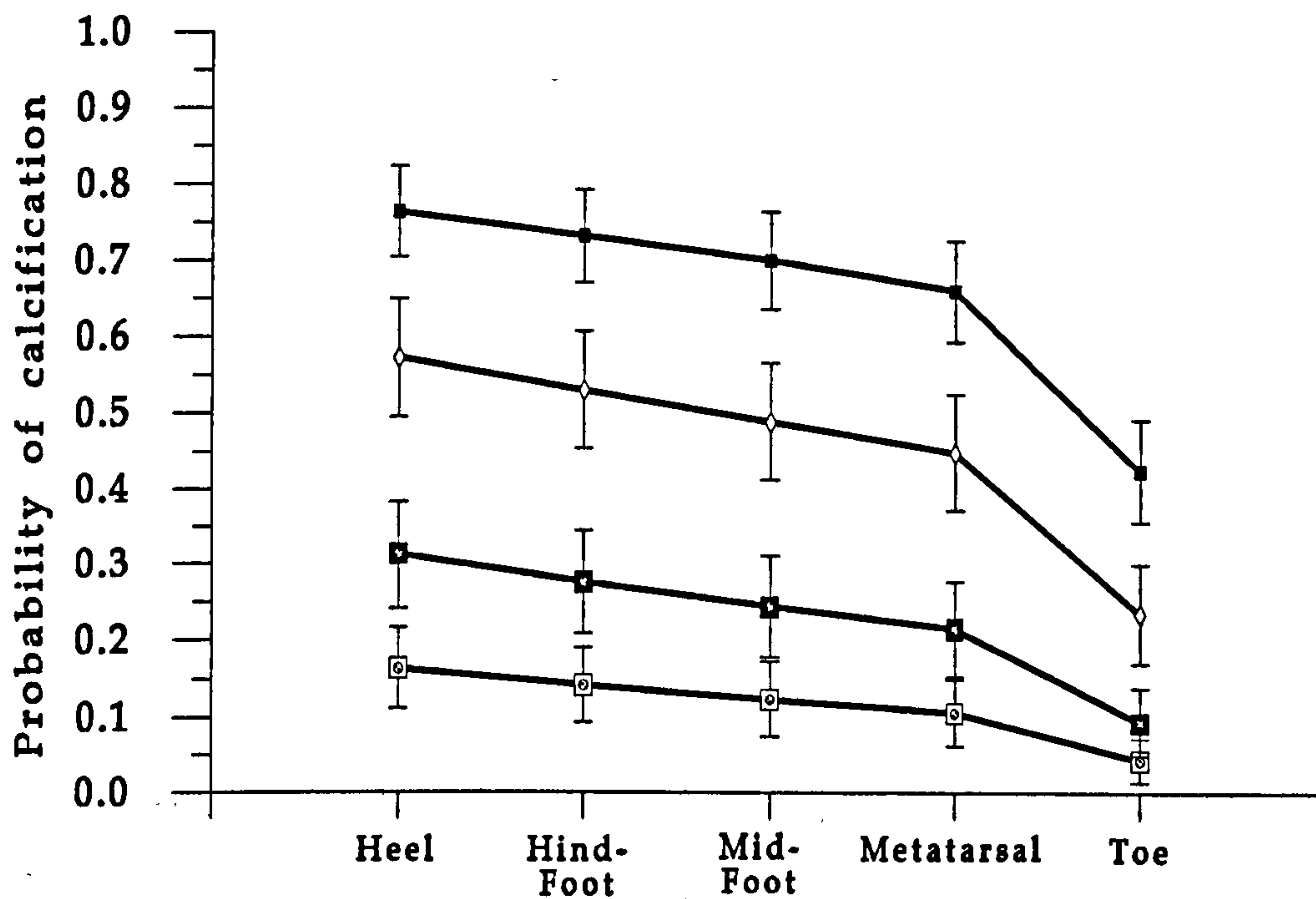


Figure 6.2. The predicted probabilities of medial arterial arterial calcification in each region of the foot for each group of subjects showing gradient from heel to toe region.

- Non-Diabetic Control subjects
- Non-Neuropathic Diabetic Group
- ◇— Neuropathic No Ulcer Group
- Neuropathic Ulcer Group

Multiple logistic regression

The presence of any calcification, either mild or severe, in a patient was predicted by two variables, VPT and duration of diabetes, where

$$\text{logodds (P(calcification))} = -2.943 + 0.069 * \text{VPT} + 0.060 * \text{duration}$$

This indicated that a 5 volt increase in vibration perception threshold increased the logodds of the presence of any calcification in the foot by a multiple of 1.42 (calculated from $\exp\{5 * 0.069\}$) and a 5 year increment in diabetes duration increased the logodds of calcification by a multiple of 1.34 ($\exp\{5 * 0.060\}$).

The presence of severe calcification in at least one region of the foot could be modelled using three variables, VPT, duration of diabetes and serum creatinine.

$$\text{logodds (P(calcification))} = -4.841 + 0.053 * \text{VPT} + 0.076 * \text{duration} + 0.012 * \text{creatinine}$$

By a similar analysis of the individual coefficients as shown in the previous model, a 5 volt increase in vibration perception threshold increased the logodds of the presence of severe calcification in any region of the foot by a multiple of 1.3, a 5 year increment in diabetes duration increased the logodds of calcification by a multiple of 1.46 and a $30 \mu\text{mol l}^{-1}$ increase in serum creatinine increased the logodds of calcification by a multiple of 1.42.

Discussion

This study found higher rates of bone and joint abnormalities in diabetic patients with peripheral neuropathy than those previously reported, especially in patients with a history of foot ulceration. Non-neuropathic diabetic patients did not appear to have any excess of abnormalities when compared to non-diabetic control subjects.

The frequency of traumatic fractures (12/54 patients - 22.2%) in the neuropathic ulcer diabetic patients was notable, particularly since 9 of these 12 patients had no recollection of - and received no treatment for - fractures. This probably reflects the profound sensory loss that can occur in these patients.

The 16% prevalence of hypertrophic Charcot changes in this otherwise unselected group of neuropathic patients with foot ulcers contrasts strongly with the value of 0.15% reported by Sinha et al (1972) in a 21 year retrospective review of 68,000 Joslin Clinic patients who were presumably of mixed neuropathic and non-neuropathic status. Although the latter figure is still frequently quoted in the literature, there are reports of higher prevalence in a variety of groups with different inclusion and exclusion criteria - including values of 7% (Pogonowska et al. 1967) and 37% (Tawn et al. 1988). These reports, and our results, suggest that the index of suspicion for Charcot changes must be high in the neuropathic diabetic patient, particularly those with a history of foot ulceration, and support the view that many such changes may have been overlooked or misinterpreted in the past (Sanders and Frykberg 1993).

The increased frequency of periosteal reaction in neuropathic patients confirms the findings of Williams et al. (1988). While periosteal reaction is sometimes regarded as evidence of response to infection (Renton 1990) it was thought by Williams et al (1988) to be a non-specific finding in the foot of diabetic patients. An alternative explanation, in the absence of foot ulceration, may be elevated mechanical stress. Animal experiments have shown a clear association between increased bending stress in long bones and periosteal reaction (Uhtoff and Jaworski 1985). Since it is now accepted that neuropathic diabetic patients have

elevated loads under the metatarsal heads (Boulton et al. 1983, Veves et al. 1991b), this could explain the increased periosteal reaction in these patients.

Although radiological evidence of bone infection was present in only one patient, 14 patients had an amputation of part or all of one foot, and since all of these had a history of plantar ulceration, infection may have been present in the majority of these cases.

The small number of atrophic or osteopaenic changes found in the present study contrasts with the frequent anecdotal reports of such findings (Kerr et al. 1991). The finding of no significant excess of abnormal joint alignment in any of the diabetic groups compared to controls is surprising. Only dislocation was significantly higher in the neuropathic ulcer diabetic group, which also had a large proportion of patients with Charcot neuroarthropathy, subluxations were approximately equal in all groups. However, clawing of the toes has been reported to be more prevalent in patients with diabetic neuropathy (Renton 1990) and the results of this study are in contrast with these previous findings. The majority of abnormalities in the control group were at the interphalangeal joints, and it may be that the reduced toe use reported in diabetic patients (Boulton et al. 1983) results in less deformity than would otherwise occur.

Other factors, in addition to neuropathy, may have played a role in the large number of bony abnormalities seen in the present sample. In particular, the high median value for serum creatinine in the patients who had experienced a plantar ulcer is a possible explanation for some of the findings of this survey, and it may be beneficial to stratify the subject groups based on renal function in future studies.

The significantly greater number of bone and joint abnormalities found in the neuropathic group should increase the index of suspicion for such changes in neuropathic diabetic patients with otherwise equivocal clinical findings. Although the increased prevalence of amputation might be expected in patients with a history of

foot ulceration, and the finding of greater degrees of periosteal reaction may have little direct clinical relevance, the prompt recognition of traumatic fractures has considerable clinical importance as it is widely believed that a traumatic fracture in a neuropathic patient can lead to Charcot neuroarthropathy. For example, in the series of 118 cases of Charcot neuroarthropathy reported by Johnson (1967) traumatic fractures were the initiating factor in 'the majority of cases'. In addition, whilst Charcot changes may only be clinically apparent unilaterally, they are bilateral on radiographs in at least 24% of cases (Sinha et al. 1972). Current clinical practice suggests that immobilisation of an early Charcot process may limit deformity (Sanders and Frykberg 1993), and in the future, the early use of intravenous Pamidronate may be used to limit the destruction of neuroarthropathy (Chapter 8). Therefore, increased surveillance, particularly of patients with peripheral neuropathy and previous Charcot changes, might allow such treatments to be started earlier in the disease process, reducing the subsequent deformity and morbidity.

This study also described a significant association between diabetes, peripheral neuropathy and the presence of medial arterial calcification. There is also a significant gradient in the prevalence of medial arterial calcification from the ankle to the toe. The association of medial arterial calcification with diabetes (Morrison and Bogan 1929, Ferrier 1964, Strandness et al. 1964, Neubauer 1971) and neuropathy (Edmonds et al. 1982, Goebel and Fuessi 1983, Everhart et al. 1988) has been described previously but there have been no previous studies of groups with different levels of diabetic complications or with age and sex matched normal control subjects using a standardised radiographic technique for each patient to allow for accurate comparisons between regions and groups.

The development of medial arterial calcification is believed to be due to autonomic denervation of the intima media of the small muscular arteries of the foot (Edmonds et al. 1982, Goebel and Fuessi 1983, Everhart et al. 1988), and increased medial arterial calcification has been reported following sympathectomy (Goebel and

Fuessi 1983). Edmonds et al. (1982) described increased vascular calcification in the knee, the hands and the feet in diabetic patients with autonomic and peripheral sensory neuropathy. In the present study the total amount of medial arterial calcification was significantly higher in the neuropathic diabetic groups than in the non-neuropathic diabetic group. The total arterial calcification score also correlated significantly with the vibration perception threshold and provides quantitative confirmation of this hypothesis.

Uncomplicated diabetes, as represented in the non-neuropathic diabetic group, did not appear to increase the development of medial arterial calcification when compared with the non-diabetic group of a similar age. This was apparent from both the total calcification score and from the statistical modelling of the calcification data but no previous study has compared uncomplicated diabetic patients with normal control subjects to confirm this.

The increase in medial arterial calcification from non-neuropathic diabetic control subjects through neuropathic patients without a history of foot ulceration to neuropathic diabetic patients with a history of foot ulceration is probably due to a combination of factors. The addition of peripheral neuropathy is associated with a significant increase in the probability of the presence of calcification and the total amount of arterial calcification, despite the fact that the non-neuropathic and neuropathic non-ulcer diabetic patients have a very similar duration of diabetes. The neuropathic group with previous foot ulceration had a higher serum creatinine and a longer duration of diabetes than the non-ulcer neuropathic group. Vibration perception threshold was not significantly different between these groups and this may be because of the proximity to the ceiling of measurable VPT and the large number of 'off scale' values in each group, 16/54 and 11/40 respectively, although the median and inter quartile range were higher in the ulcer group. A chi-square test of presence of calcification with an off-scale VPT was also highly significant, further reinforcing the association of medial arterial calcification and neuropathy

The combination of factors, serum creatinine, duration of diabetes and VPT, all of which significantly correlated independently with the total calcification score and strongly predicted the presence of severe calcification in the multiple logistic regression model, may have contributed to the higher levels of arterial calcification within the neuropathic ulcer group. It is not however possible, from the present analysis to infer that the three predictor variables are independent risk factors for the probability of the presence of severe arterial calcification.

The lack of any correlation with age and calcification is surprising. Increasing age is associated with an increase in medial arterial calcification in a number of studies (Morrison and Bogan 1929, Ferrier 1964, Strandness et al. 1964, Neubauer 1971, Goebel and Fuessi 1983). A possible explanation is the high median and narrow range of age of our subjects. Three-quarters were aged over 50 years, at which time calcification was more common in all these studies. This may have masked any possible relationship of calcification with age. However the non-diabetic control group were well matched to the diabetic group in age and yet they had virtually no arterial calcification.

The higher total arterial calcification score in type 1 (insulin-dependent) diabetic patients in each group is probably due to the longer known duration of diabetes in these patients compared to type 2 (non-insulin-dependent) diabetic patients. This is in keeping with a report of higher ankle-brachial pressure indices in type 1 (insulin-dependent) diabetic patients, which was attributed to higher levels of medial arterial calcification and a longer duration of diabetes (Goss et al. 1989). This was not reflected in an increase in the prevalence of any degree of calcification in type 1 (insulin-dependent) diabetic patients as demonstrated by the fact that diabetes type did not enter the logistic regression model.

The probability of calcification in each group suggested a trend from non-neuropathic diabetic patients to ulcer diabetic patients and from the toe to the ankle region in each group. The ordered categorical model (Agresti 1990) allows further

analysis of repeated ordinal measurements by suppressing the underlying variability of these measurements. The increased probability of medial arterial calcification in the neuropathic ulcer group confirmed the trend observed in the raw data, which had indicated that the probability of medial arterial calcification was greater in the neuropathic ulcer group than in the neuropathic diabetic patients without a history of foot ulceration. It also confirmed the increase in calcification in neuropathic patients compared to non-neuropathic patients, and also that diabetes alone, when not complicated by neuropathy, did not add to the prevalence of calcification found in age and sex matched control subjects.

The ordered effect of region, which represents the increase in calcification progressing from the metatarsals to the ankles provided a good linear fit of predicted prevalence vs observed prevalence. However the prevalence of medial arterial calcification at the toe in all groups was significantly lower than that predicted by a purely linear model. This 'toe effect' was estimated and when introduced as a factor of 0.42 times the probability of calcification for a linear fit, made the model more representative of the data.

The testing of the raw prevalence data with a statistical model has confirmed the inter-group and inter-regional trends which would have been difficult to test significantly using individual statistical tests. These trends are also clinically relevant.

Screening diabetic patients for peripheral vascular disease and implied cardiovascular disease (Nillson et al. 1967, Carter 1969, Yao et al. 1969, Neubauer 1971, Cutajar et al. 1973, Janka et al. 1980, Goebel and Fuessi 1983) often includes a measurement of the ankle brachial systolic pressure index (Carter 1969, Yao et al. 1969, Cutajar et al. 1973, Janka et al. 1980). This measure has however been discredited by the false elevation reported in diabetic patients with non-compressible medial arterial calcification in the tibial and ankle plexus arteries (Reimann and Bollinger 1974, European Consensus 1989). Ankle systolic pressure and ankle-brachial pressure index were significantly associated with total arterial calcification

score in this study, and, as calcification was more marked in the ankle and hind foot regions this would support this assumption. However, whilst an ankle systolic blood pressure of >200 mmHg is often believed to demonstrate the presence of medial arterial calcification, this study demonstrates that arterial calcification is present in 8.6% of patients with ankle systolic pressures below 100 mmHg. Carter (1973) investigated the effect of medial arterial calcification on intra-arterial measurements of dorsalis pedis arterial pressure in five patients. One patient had a significant rise in cuff pressure versus directly measured arterial pressure, whilst the others had slightly lower cuff pressures. Similar results were found in patients with brachial artery calcification. However, this study was uncontrolled and requires further studies to be performed to confirm the main findings, particularly as they appear to contradict clinical and non-invasive testing experience (Cutajar et al. 1973, Reimann and Bollinger 1974, Everhart et al. 1988, European Consensus 1989, Goss et al. 1989). It is therefore quite likely that in diabetic patients, ankle systolic pressures and ankle-pressure indices may be falsely elevated even at 'normal' levels due to medial arterial calcification. For this reason, toe systolic pressure is regarded as a more reliable indicator of arterial inflow to the foot than ankle pressure in diabetic patients (European Consensus 1989). The demonstration of significantly lower levels of arterial calcification in the toe regions provides a possible explanation for this premise and a rationale for the use of toe, rather than ankle, pressure measurements in diabetic patients. However, this hypothesis remains to be tested.

Conclusions

In general, the results of these studies clearly demonstrate the increased prevalence of clinically important bone and joint abnormalities in diabetic patients with peripheral neuropathy, particularly in association with foot ulceration. They also suggest that many of the minor changes, including misalignments and medial arterial calcification, previously thought to be more common in diabetic patients regardless of the presence or absence of complications, may in actual fact be equally as common in the general population.

The high prevalence of Charcot neuroarthropathy in this survey should increase the index of suspicion for such changes in neuropathic patients.

In addition this study, using standardised methods to compare well defined groups, has confirmed the previous assumptions about the associations of medial arterial calcification and neuropathy, duration of diabetes and renal dysfunction. The low discriminatory value of a defined level of ankle pressures to infer the presence of arterial calcification and the converse, that calcification may be elevating the ankle-pressure index, even in the normal range, must cast further doubt over the use of ankle pressures to screen for lower limb arterial disease particularly in diabetic patients with neuropathy. The demonstration of a gradient in calcification between the ankle and the toes supports the theoretical use of toe pressure measurements rather than ankle pressures in diabetic patients, but this benefit remains to be proven clinically.

Section Four

Studies of Patients with Charcot Neuroarthropathy

Chapter Seven

Neurophysiological and Bone Mass Studies in Patients with Charcot Neuroarthropathy

Patients

Two groups of patients were studied to determine the predisposing factors associated with the development of Charcot neuroarthropathy, and whether there was a qualitative difference in the degree of neuropathy present in patients with Charcot feet. The Charcot patients comprised 17 consecutive patients attending the diabetic foot clinic of the Manchester Foot Hospital presenting with radiologically proven active Charcot neuroarthropathy. Charcot change was defined using the criteria of Cofield et al. (1983), as the simultaneous presence of bone and joint destruction, fragmentation and remodelling as in Chapter 6. Charcot activity was defined as a temperature difference of $>2^{\circ}\text{C}$ between the active and non-active foot (Sanders and Frykberg 1991).

These patients were then compared with 17 age, sex and duration matched patients with clinical neuropathy, as recorded on the computerised database of the Manchester Diabetes Centre. All non-Charcot patients had normal weight bearing radiographs of the feet and no clinical evidence of Charcot change.

The physical characteristics of the subjects are listed in Table 7.1.

Table 7.1. Physical characteristics of the patients included in the study of the differences in neurological deficit between Charcot and non-Charcot diabetic patients with neuropathy.

	Charcot	Non-Charcot
Number	17	17
Female : Male	11 : 6	7 : 10
Age (years)	49 ± 13	54 ± 10
Duration of diabetes (years)	20 ± 9	19 ± 11
Type I:II	10:7	10:7
Duration of Charcot (years)	1.35 ± 1.02	
HbA1 (%)	9.9 ± 1.6	9.4 ± 2.9
Creatinine (μmol l ⁻¹)	130 ± 62	120 ± 98
BMI (kg m ⁻²)	28 ± 6	27 ± 3
Previous Foot Ulcer	10	9
Retinopathy*	11	11
Proteinuria**	8	7

Results are shown as mean ± SD, or absolute number. There were no significant differences between groups.

* defined as background or worse on dilated fundoscopy.

** defined as dipstix positive.

Methods

Both groups of patients had the following tests of neurological function: neuropathy deficit score, vibration perception threshold, warm and cool thermal perception thresholds, peroneal nerve motor conduction velocity, and cardiovascular autonomic function, performed as described in Chapter Two (Methods), pages 53-57.

Skin temperature was also measured using the Mikron infra-red thermometer (Methods, page 60).

The patients Charcot neuroarthropathy also had bone mass measurements by single photon absorptiometry and dual energy X-ray absorptiometry to determine if there was a reduction in skeletal mass to account for the increased fracture risk as a predisposing factor in the development of Charcot neuroarthropathy (Methods, page 78).

Statistical Analysis

The neurophysiological results were compared as Charcot foot versus non-Charcot foot in the Charcot patients, and Charcot foot versus the mean of the results for both feet in the non-Charcot patients, using standard comparative statistics (Mann-Whitney U tests, Wilcoxon Rank Sum tests and t-tests where appropriate).

Results

Neurological Function

The results are detailed in Table 7.2. In summary, there were no significant differences in all the measures of neuropathy between the affected and non-affected foot in Charcot patients.

The neuropathy disability score (median 10 (10-10 interquartile range) was significantly worse in the Charcot patients compared to the non-Charcot patients (median 8 (7-10)), $p=0.009$.

Peroneal nerve motor conduction velocity was significantly worse in the Charcot patients versus the non-Charcot patients (30.0 ± 4.8 vs. 33.3 ± 3.5 ms⁻¹, $p=0.010$).

Vibration perception threshold and warm perception threshold were higher, but not significantly, in the Charcot patients compared with the non-Charcot patients. Cool perception threshold was similar in both groups (Table 7.2).

Autonomic function was abnormal in all the Charcot patients, but in only 10 of the non-Charcot patients ($p=0.03$).

Skin temperature was significantly higher in the active Charcot foot than in the non-Charcot foot by definition (32.8 ± 1.8 vs. 29.6 ± 2.6 °C, $p=0.8$). There was no significant difference between the non-Charcot foot and the skin temperature of the feet of non-Charcot patients (29.6 ± 2.6 vs. 29.6 ± 1.7 °C, $p=0.8$).

Table 7.2. Results of neurophysiological tests in Charcot and non-Charcot patients.

	Charcot foot	Non-Charcot Foot	Non-Charcot Neuropathic Patients
Neuropathy disability score	10 (10-10)		8 (7-10)*
Peroneal nerve MCV (ms ⁻¹)	30.0 ± 4.8	30.3 ± 3.8	33.3 ± 3.5**
VPT (Volts)	40.4 ± 13.0	40.4 ± 13.0	33.9 ± 13.2
Warm Thermal Threshold (°C)	10.4 ± 3.0	10.2 ± 6.2	8.2 ± 7.2
Cool Thermal Threshold (°C)	7.0 ± 5.0	5.4 ± 2.2	6.5 ± 5.9
Skin Temperature (°C)	32.8 ± 1.8	29.6 ± 2.6	29.6 ± 1.7
Abnormal autonomic function	17/17		10/17***

Results are shown as mean ± SD, except for neuropathy disability score (median (interquartile range)) and abnormal autonomic function (number of patients with abnormal tests).

* p = 0.009 Non-Charcot vs. Charcot

** p = 0.010 Non-Charcot vs. Charcot

*** p = 0.03 Non-Charcot vs. Charcot

Bone Mass Measurements

Table 7.3. Results of bone mass measurements in patients with Charcot neuroarthropathy. Results are shown as Z scores of deviance from the mean of age, sex and weight matched healthy control subjects. Scores of -1 or less indicate increased fracture risk (SPA - single photon absorptiometry, DEXA - dual energy X-ray absorptiometry).

Charcot patients

	SPA		DEXA		
	Distal radius	Proximal radius	Spine	Right and Left Femoral Necks	
Mean Z Score	-0.84	-0.53	-0.10	-1.01	-1.03
SD	1.27	1.31	1.70	2.04	2.07
Median	-0.50	-0.65	-0.40	-1.70	-2.00

The results show a marked loss of bone mineralisation in the cortical bone of the lower limbs.

Chapter Eight

A Study of Pamidronate as a a Possible New Treatment for Diabetic Charcot Neuroarthropathy

Patients and Methods

The last eight consecutive patients from the studies of neurophysiological and bone mass changes (described in Chapter Seven), attending the Manchester Diabetes Centre with typical changes of active Charcot neuroarthropathy, were studied to assess the potential of Pamidronate (Aredia, Ciba-Geigy) to halt to progression of the Charcot process.

The subjects comprised five women and three men with a mean age of 37.1 ± 19.9 (SD) years. Full details of the subjects are given in Table 8.1; they all had long standing diabetes mellitus and a variety of long term complications including in every case, a dense peripheral sensory neuropathy and autonomic neuropathy. In each case the presence of Charcot neuroarthropathy was initially suspected on the basis of the clinical picture of a swollen uncomfortable hot foot in the absence of any evidence of infection. The diagnosis of Charcot neuroarthropathy was confirmed by the simultaneous presence of bone destruction and fragmentation, with joint destruction or subluxation on examination of weight bearing radiographs of the feet as in the previous studies in this thesis (Chapters 6,7). Activity was confirmed by increased bone turnover on three phase scintigraphy using ^{99m}Tc -hydroxymethylene bisphosphonate (Edmonds et al. 1985). If there was significant clinical doubt as to the absence of infection this was confirmed by scintigraphy using radiolabelled leukocytes (Hetherington 1982).

Table 8.1. Physical characteristics of the Charcot patients receiving Pamidronate treatment.

Number	8
Female : Male	5 : 3
Age (years)	46 ± 13
Duration of diabetes (years)	20 ± 9
Type I:II	5 : 3
Duration of Charcot (years)	0.8 ± 0.4
HbA1 (%)	9.1 ± 1.0
Creatinine (μmol l ⁻¹)	141 ± 90
BMI (kg m ⁻²)	25 ± 5
Previous Foot Ulcer	5
Retinopathy*	4
Proteinuria**	3

All results are shown as mean ± SD or as absolute numbers.

*defined as background or greater retinopathy on dilated fundoscopy.

** defined as dipstick positive proteinuria.

Methods

Each subject was treated with intravenous pamidronate as described in the methods, Chapter 2, page 79.

The activity of the Charcot process was monitored by measuring the temperature of the affected foot with a Mikron infra red thermometer (Mikron Instrument Company, Inc., Wyckoff, New Jersey, USA) as described in the methods page 60. Measurements were taken immediately prior to each infusion and two weeks following the final infusion. The normal difference between the feet does not exceed 2 °C (Sanders and Frykberg 1991). At each visit blood was drawn for the measurement of alkaline phosphatase activity and creatinine concentration. Patients were also asked to describe their symptoms of pain, swelling and immobility.

Statistical analysis

Statistical analysis was undertaken using the SPSS/PC program. Analysis of variance was used to seek differences in each of the variables measured with time, with the subsequent use of Duncan's range test to identify differences in the means of these variables.

Results

Tolerability

Apart from a minor pyrexial reaction in two subjects the pamidronate therapy was well tolerated. Neither subject requested that the treatment be discontinued, nor was the reaction felt significantly serious enough to require an end to their participation. The patients' descriptions of their symptoms did not lend themselves to quantification but all subjects reported a marked decrease in pain and swelling so that their mobility was markedly improved. In the three patients who received treatment first this clinical improvement has been maintained for over a eighteen months after the completion of the course of pamidronate infusions. There was no change in renal function during the course of treatment.

Activity Measurements

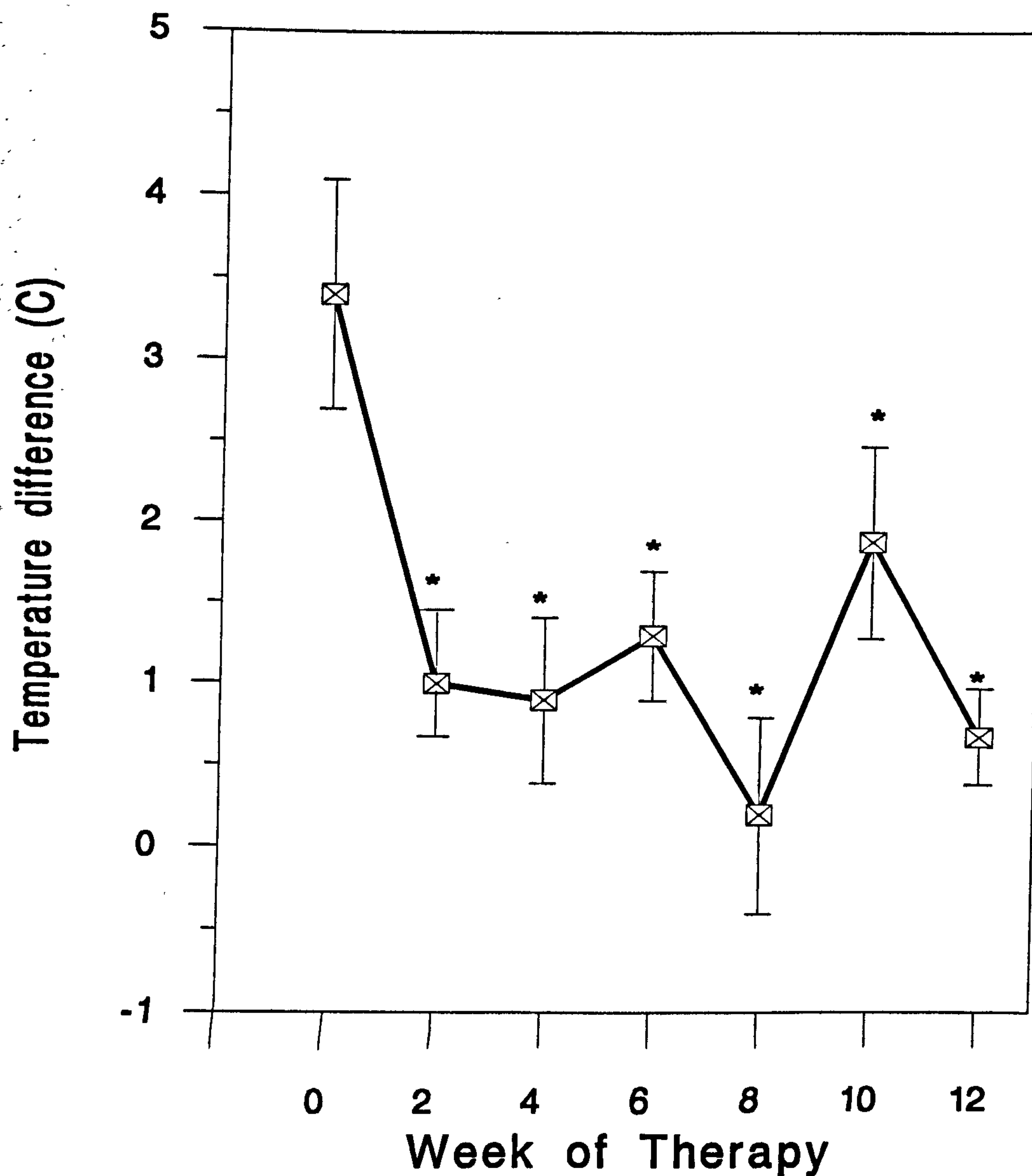
Skin Temperature

From a basal temperature difference of 3.4 ± 0.7 (SE) °C between the affected and intact foot there was a rapid fall to 1.0 ± 0.5 °C after the initial 30mg infusion (Figure 8.1). Throughout the rest of the period of observation the temperature difference remained within the normal range (within 2 °C of other foot). The overall temperature change was significant ($F=2.32$; $p=0.05$) and the pretreatment values were significantly different from those obtained at all other time points.

Alkaline phosphatase

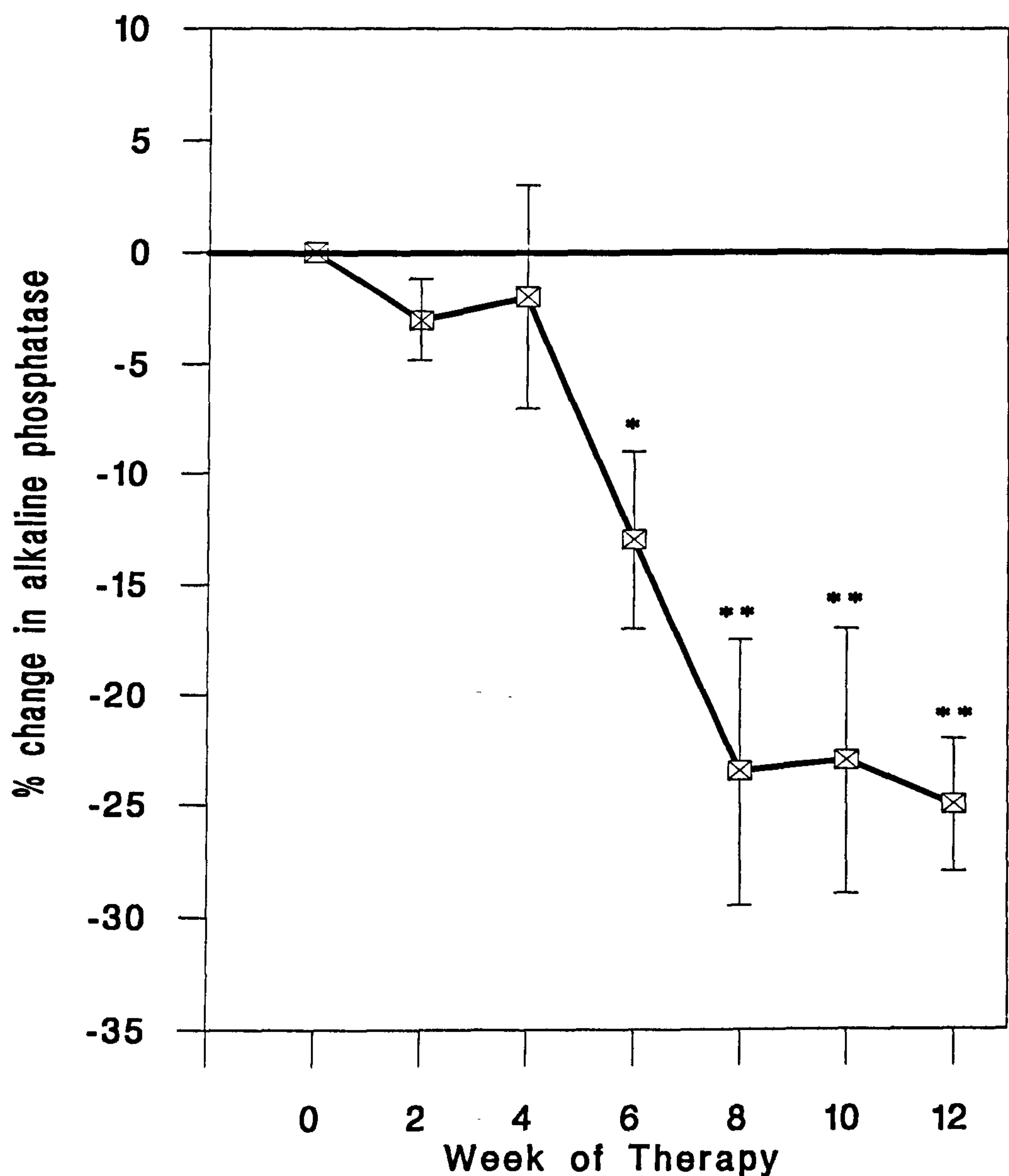
To remove the variation introduced by the wide scatter of initial values for alkaline phosphatase activity (273-503 units l^{-1} : reference range 70-330 units l^{-1}) subsequent results were expressed as a percentage of the initial value for each individual (Figure 8.2). There was a steady decline in alkaline phosphatase activity after the third infusion, such that it had reached 75% of its initial value after the final infusion. This change was highly significant ($F=8.5$; $p=0.001$) and was significantly different from baseline at all points subsequent to the third infusion.

Figure 8.1. Change in skin temperature difference between the Charcot and non-Charcot foot during treatment with Pamidronate. Temperature difference shown as mean + SE.



* $p < 0.05$ vs. initial temperature difference

Figure 8.2. Graph showing the change in mean alkaline phosphatase activity from pre study level in the Charcot patients treated with Pamidronate.



* $p < 0.01$ vs. initial alkaline phosphatase.

** $p < 0.001$ vs. initial alkaline phosphatase.

Chapter Nine

Discussion of Results in Section Three

Discussion

Charcot neuroarthropathy is a severe complication of diabetes. Taken together with previous published studies (Pogonowska et al. 1967, Tawn et al. 1988), the study in Chapter 6 suggests that it is more common amongst neuropathic diabetic patients than previously recognised (Sanders and Frykberg 1993). The aetiology of Charcot neuroarthropathy is also poorly understood. The studies in Chapter 7 dispute the recent suggestion that there is a specific neuropathy in diabetic patients with Charcot neuroarthropathy (Stevens M et al. 1992b), but support the more traditional view of patients with Charcot feet having a dense peripheral sensorimotor neuropathy associated with a long duration of diabetes (Sanders and Frykberg 1993), and the universal presence of cardiovascular autonomic dysfunction (Edmonds et al. 1985). This autonomic dysfunction is believed to lead to arterio-venous shunting in the feet of neuropathic diabetic patients, increasing the vascularity of the bone and precipitating the rapid destruction of bone following a (minor) traumatic event (Edmonds et al. 1985). It is also possible that this increased vascularity leads to reduced bone mineralisation, such as that found in the lower limbs of the Charcot patients measured in these studies. Neuropathy has been previously suggested by Edmonds et al. (1985), and Selby (1988), to be one of the causes of osteoporosis in diabetic patients. The combination of insensitivity to pain and reduced bone mineralisation may then explain the clinical syndrome of acute Charcot fractures following relatively trivial injury (Sanders and Frykberg 1993, McEnery et al. 1993).

None of the present treatments for Charcot neuroarthropathy has a significant effect on the underlying process of bone destruction. The results of this study suggest that pamidronate may be able to fulfil this role. Even in the absence of a placebo treated group it would appear highly unlikely that the activity of a Charcot joint would diminish spontaneously within two weeks of infusion of pamidronate in eight separate

patients, whose disease process had been highly active and unchanged for several weeks, and in most for up to a year, prior to treatment. Nevertheless, such a controlled trial may need to be performed. An additional benefit of Pamidronate is that it may also treat any underlying osteoporosis and possibly reduce the risk of further fractures.

Temperature changes are widely accepted as being an appropriate means of assessing the activity of the Charcot process (Sanders and Frykberg 1991), and given the profound changes observed in skin temperature of the foot and alkaline phosphatase in the patients studied, together with the subjective improvements in symptoms, there is little doubt that the Charcot activity was been markedly reduced during the treatment period. The change observed in alkaline phosphatase was not due to improved diabetic control, since there was no significant change in this over the period of study. There have been few studies of the bisphosphonates in normal volunteers but one such experiment suggested that the expected change in alkaline phosphatase activity after administration of pamidronate to volunteers without bone disease was only 10% (Netelenbos et al. 1991). It would have been desirable to obtain a further measure of disease activity such as repeated isotope scans, but it proved impossible to obtain sanction for this from the Administration of Radioactive Substances Advisory Committee in time to complete these studies.

The pamidronate regimen used in this study was based upon the use of a protocol which has proved successful in Manchester in the management of patients with Paget's disease of bone (Anderson et al. 1993). This is by no means the only possible regimen and recent research has suggested that a variety of different protocols, some of them based on single infusions of bisphosphonate, are equally effective in the management of Paget's disease (Hooper et al. 1993, Watts et al. 1993) and so may well be equally effective in the treatment of Charcot neuroarthropathy giving that a reduction in skin temperature was apparent after the first half dose

(30mg) infusion. In the treatment of Paget's disease with pamidronate the regimen of Anderson et al. appears to be effective at suppressing disease activity for between six and eighteen months, following which there is a similar response to repeated therapy in patients who relapse (Anderson et al. 1993). There is no obvious reason to believe that a similar disease free interval could not be expected in patients with Charcot neuroarthropathy. Indeed, five of the initial patients have been maintained in clinical remission for over one year, and no patient has yet relapsed.

It is generally held that Charcot neuroarthropathy is a rather rare complication of diabetes, whilst this is undoubtedly true for the relatively late stages of the process with gross deformity and fracture (Sinha et al. 1972), this is not so for less severe changes. The survey of radiological findings in diabetic patients described in Chapter 6, and work by others (Cofield et al. 1983) would suggest that early signs of neuroarthropathy are present radiographically in up to 10% of diabetic patients with neuropathy, which would suggest that the complication might be present in up to 2% of people with diabetes. With increased awareness of the early stages of the Charcot process, including the management of otherwise trivial fractures (Johnson 1967), along with expertise in the use of bisphosphonates, particularly following a controlled trial, it might be possible to treat patients at much earlier stage of the disease and hence avoid the later development of bony deformity and subsequent morbidity due to ulceration.

Conclusions

In conclusion, patients with Charcot neuroarthropathy have been shown to have reduced bone mass and a dense global peripheral neuropathy. These, together with autonomic neuropathy are in keeping with current theories for the aetiology of Charcot foot as a neurotraumatic process (Sanders and Frykberg 1993).

Pamidronate, unlike any previous therapy, appears to moderate the underlying bone destruction inherent in the active phase of the Charcot process. It is therefore possible that bisphosphonates may have an important future role in the prevention of the progression of this distressing condition.

Section Five

Final conclusions
and references

Conclusions

1. Rheological parameters are significantly abnormal in diabetic patients with neuropathy, and adversely altered, although not significantly in this study, in non-neuropathic diabetic patients. These changes might lead to increased formation of capillary microthrombi due to increased red cell aggregation and increased fibrinogen concentrations. Taken together with impaired fibrinolysis, leading to reduced clearance of such microthrombi there is evidence that microvascular flow might be impaired in neuropathic patients. The inverse correlations between transcutaneous oxygen and rheological parameters support the view that they might influence microvascular flow.
2. The finding that transcutaneous oxygen levels are significantly reduced in neuropathic patients is in keeping with other studies and provides further evidence for microvascular impairment in peripheral neuropathy.
3. The improvement in peroneal conduction velocity with improved tissue oxygenation following revascularisation of the legs of non-diabetic and diabetic patients with peripheral vascular disease strongly suggests that endoneurial hypoxia due to whatever cause has an adverse effect on peripheral nerve function and may be implicated in the aetiology of peripheral neuropathy.
4. Diabetes alone does not seem to increase the number of skeletal and soft tissue abnormalities found on radiographs of the feet when compared to age and sex matched non-diabetic control subjects.
5. Neuropathy, particularly in association with foot ulceration, is however associated with a marked increase in skeletal abnormalities, including fractures, most of which were unknown to the patient, medial arterial calcification, and Charcot changes.
6. Medial arterial calcification is so common in neuropathic diabetic patients that ankle systolic pressures are likely to be unreliable in the majority of patients.

However, they are unlikely to be as unreliable in non-neuropathic diabetic patients.

7. The demonstration of a gradient in the prevalence of calcification between the ankle and the toes would suggest that toe pressure measurements might be more reliable than ankle pressures in neuropathic diabetic patients, but as yet there is no clinical evidence to support this.
8. Charcot neuroarthropathy is probably more common than widely recognised.
9. Charcot patients appear to have a severe global neuropathy; in keeping with the generally held view that it is the densely neuropathic patients who develop Charcot changes.
10. Charcot patients have a reduced bone mass in the lower limbs, and this might be part of the reason why they develop fractures after minor trauma which then start the Charcot process.
11. Pamidronate is an effective treatment for acute Charcot neuroarthropathy, reducing the disease activity and relieving the physical symptoms. It may also help to improve the low bone mass found in Charcot patients.

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